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CHEMOTHERAPY

OF

ANIMAL TRYPANOSOMIASIS

A Résumé of Literature)

By

S. K. SEN, D.Sc.

Formerly Research Officer, Indian Veterinary Research Institute, **Mukteswar**

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ANIMAL TRYPANOSOMIASIS

(A Résumé of Literature)

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2	8	feelings	feels
2	37	(suramin)	(suramin),
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7	19	for cattle	for cattle,
7	42	was	is
8	41	mortility	mortality
9	18	suramin although	suramin, although
11	1	abeess	abscess
13	12	alcchol get	alcohol, get
13	20	‘*T. equiperdum*’	*T. equiperdum*’
13	24	fluid. Yorke	fluid, Yorke
13	38	d ugs	drugs
17	40	‘gufar’	*gufar*
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17	42	po itive	positive
19	25	test i neffective	test ineffective
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27	36	co-efficient	coefficient
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29	31	cseas	cases
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38	11	curr-	cura-
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42	35	‘souma’	*souma*
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43	9	atxoyl	atoxyl
43	15	inncculated	inoculated
43	19	‘*jaffa*’.	*jaffa*
43	19	‘*el debab*’	*el debab*
43	19	‘*el zombab*’	*el zombab*
43	22	the doses, being	the doses being
43	26	‘*souma*’	*souma*
43	26	‘*baleri*’	*baleri*
43	27	‘*m’bori*’	*m’bori*
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24	'Four eau 270	'Fourneau 270'
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35	with	by
38	two-fold	twofold
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4	T RTAR	TARTAR
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17	1 gm. for a year....donkeys	1·5 gm for adult bo
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19	months the	months; the
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45	8 kilo., bodyweight	8 kilo. bodyweight
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4	Suramin	suramin
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Page	*Line*	*for*	*read*
176		*Delete* the first three lines '*Ferreira (1948). *The Trypanosomiasis*........**46**, 530; 1949]'	
176	20	Senior (1921).	Senior—
176	20	*Proc., 4th entom.*	*Proc. 4th entom.*
176	21	White.	White (1921).
179	13	*Abt.* 1	Abt. **1**
179	26	*—[1914]	—(1914).
180	12	*Mem., Dep. Agric, India,*	*Mem. Dep. Agric. India,*
180	16	*Mem. Dep., Agri. India vet.*	*Mem. Dep. Agric. India, vet.,*
182	2	*Arch. Insts. Pasteur*	*Arch. Insts Pasteur*
184	8	*Immun Forsch.*	*ImmunForsch.,*
186	15	*Ann. Pharm. France*	*Ann. Pharm. Fr.,*
186	20	*Bull. Soc., Path.*	*Bull. Soc. Path.*
189	foot-note	not examined.	not examined
190	12	*Gesundh Amt.,*	*GesundhAmt.,*
190	33	*Immun Forsch.,*	*ImmunForsch.,*
194	29	*J. infec. Dis.*	*J. infect. Dis.*
197	13	*Immun Forsch*	*ImmunForsch*
197	23	*Immun Forsch*	*ImmunForsch*
197	35	*Immun Forsch*	*ImmunForsch*
198	1	*Immun Forsch*	*ImmunForsch*
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198	5	*Immun Forsch*	*ImmunForsch*
200	17	3, 1924	3; 1924
200	19	**12**, 1924	**12**, 113; 1924
200	21	**47** 449	**47**, 449
201	5	*Pasteur, Algere,*	*Pasteur Algér,*
202	5	*Gold Cst. 1931-32*	*Gold Cst, 1931-32*
202	10	*Gold Cst. 1947*	*Gold Cst, 1947*
202	25	*J. infec. Dis.*	*J. infect. Dis.*
204	23	*Pasteur Tunis*	*Pasteur, Tunis*
205	1	*Path exot.*	*Path. exot.*
205	25	1939].	1939]
207	2	*Soc., Path.*	*Soc. Path.*
207	22	*exot.* **18**	*exot.,* **18**
207	24	*vet., Bull.*	*vet. Bull.*
209	14	*vet. Bull.* **5**	*vet. Bull.,* **5**

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PREFACE

IN 1928, the late Dr. J. T. Edwards, Director, Indian Veterinary Research Institute, Mukteswar, published his well-known memoir entitled 'The Chemotherapy of Surra (*Trypanosoma evansi* Infection) of Horses and Cattle in India',* which also appeared in abridged forms in *Journal of Comparative Pathology and Therapeutics* and in *Transactions of the Royal Society of Tropical Medicine and Hygiene.* The compiler of the present monograph had the privilege of being entrusted with the drafting of outline of the memoir on the basis of a considerable volume of charts and protocols of experiments carried out by Dr. Edwards during 1923-25. In the course of a very generous acknowledgment (*vide* p. 54 of the memoir) of what assistance the compiler was able to render him in preparing the memoir, Dr. Edwards made the statement that a résumé of literature on the chemotherapy of trypanosomiasis would form the subject of a separate publication by the compiler of this monograph. It is now nearly three decades since that statement was made but, on account of more pressing demands on his time, the compiler has so long been unable to undertake the preparation of the résumé. It is a melancholy satisfaction for him that he has at long last been able to discharge his moral obligation to Dr. Edwards—melancholy because he died in England in 1953 without seeing the MS of the monograph, so that it has now assumed the position of a posthumous addendum to his memoir. He can now only derive some consolation from the thought that the preparation of this monograph has been a vicarious undertaking for him, for the work would have been more appropriately done by Dr. Edwards himself.

Some 1800 references covering a wide range of problems in the field of chemotherapy have been consulted in compiling this monograph. The textual matter relating to the more important drugs has been divided into 14 chapters, commencing, as is customary, with arsenicals amongst the metallic compounds and ending with antrycide, the most recent introduction in the group of non-metallic compounds.

The inclusion of arsenophenylglycin in the list of drugs dealt with in the monograph calls for a word of explanation. The discovery of this arsenical by Ehrlich created at one time a degree of optimism which is only equalled by the introduction of such recent drugs as suramin and antrycide. Apart from its historical interest, arsenophenylglycin represents a class of drugs which, by reason of their being very unstable, may operate in a manner entirely different from the mechanism of action of stable compounds.

Atoxylization, which enjoyed so much popularity in the early days of chemotherapy as a means of dealing with both sleeping sickness and animal

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* *Mem. Dep. Agric. India, vet.,* 4, 1—100

INTRODUCTION

TRYPANOSOMIASIS, whether it affects man or animals, constitutes one of the most baffling problems that have confronted the medical and veterinary profession. Since Ehrlich developed his classical conception of the science known as 'Chemotherapy'—as distinct either from pure therapy or pharmacology—all manner of ingenuity, bewildering in its complexity, has been brought to bear upon the question of dealing with this formidable disease and specificity has been claimed for remedies brought into the field of Chemotherapy from time to time after intensive trials in the laboratory. But such claims have only been short-lived, having had to be eventually sacrificed at the altar of drug-fastness. This remark applies even to such well-known remedies as atoxyl, tryparsamide, suramin and antrycide. One thus notices that, in spite of half a century's* well directed campaign against sleeping sickness in Belgian possessions in Africa by missionary and other organizations, the following announcement appeared as recently as in October 1952: 'The Belgian Government has offered a reward of 1 million Belgian francs for the discovery of an effective and lasting check for sleeping sickness largely prevalent in the Belgian Congo, according to a press release from the Belgian Consulate-General here [in Calcutta].

'A jury, consisting of three doctors, specialists in tropical diseases and presided over by a layman will examine whether the proposed remedies fulfil the necessary conditions for the radical cure of the disease.

'The Belgian Government had offered in 1906 a reward of 200,000 francs for the same purpose but there had been no successful discovery, it was stated.

'The award is open to all irrespective of nationality.'

The compiler of this monograph has endeavoured to make a correct appraisal of the view points, advanced by different workers during the past 45 years, concerning the extent to which it may be possible to combat trypanosomiasis and it is with a feeling of profound disappointment that he has been led to the conclusion that, while drug intervention is, as a rule, successful when applied in the early stages of trypanosomiasis, it has in the advanced stages of the disease to be regarded as refractory to all known methods of treatment, for the simple reason that, in prolonged cerebrospinal involvement, the meninges suffer a certain degree of damage which is not amenable to any known form of repair. This remark also applies to equine surra in India, where the destruction of animals as

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*It would appear to be disputed when exactly the causal parasite of sleeping sickness was recognized. According to Kleine (1936), Castellani noticed the association of trypanosomes with sleeping sickness, but their significance as the causative agents of the disease was first recognized by Bruce, so that the latter should be regarded as the real discoverer, although 'even now several authors do not realise that it was David Bruce who was the real discoverer'. In a rejoinder published in 1939, Castellani quoted a letter written by him in 1903 in evidence of his claim that Castellani himself discovered the cause of the disease. He cited the names of several authorities, including Ross, as having 'always given me the credit' for the discovery in the elucidation of the aetiology of sleeping sickness. Cook (1941), in complete agreement with Klein, states that little was known up to 1900 as to how sleeping sickness was caused and Manson even suggested *Filaria penetrans* as its causal parasite. He further states that Castellani, presiding over the Sleeping Sickness Commission, detected trypanosomes in the cerebrospinal fluid of patients but regarded it as of no significance. According to him, Bruce, in 1904, not only attributed the disease to trypanosomes, but also obtained evidence of its transmission through *Glossina palpalis*. The fact that Castellani was the earliest to record trypanosomiasis has evidently led some workers to use the generic name *Castellanella* in preference to *Trypanosoma*.

required by the Glanders and Farcy Act would still appear to be the only method of dealing with late cases of the disease. In recording this pessimistic note, the compiler cannot help paying a tribute to the many missionary workers, as also medical and veterinary men, who, at considerable risk to their lives, have waged unrelenting campaign in Africa against the disease and its vectors. The story of their spirit of service and devotion to duty makes most stimulating reading and will continue for all times to be an incentive for those who have the good of mankind at heart.

One feelings struck by the fact that the entire literature on the subject of chemotherapy of trypanosomiasis is punctuated by notes of defeatism expressed by different workers as to the prospect of any known form of therapeutic measure proving effective against all stages of the disease. Thus, Raven (1909) does not advise the use of arsenophenylglycin—which was at that time regarded as almost a specific for sleeping sickness—in advanced cases requiring prolonged treatment. It was evidently in despair that Fusco (1909) stated that trypanosomiasis was incurable and that arsenic—according to him the most efficacious remedy of the time—could at the best prolong life. In a paper published in 1911, Gaiger in India presented a discouraging picture of surra as it existed in those days. He pointed out that the several cases of equine surra claimed by Holmes, during 1909-10, as having been cured by him by the use of arsenicals, were all artificial and that there was not one record of recovery having been obtained in the treatment of the natural disease. He considered measures against vectors—unfortunately these are not definitely known even to this day—and the slaughter of carriers (such as cattle) as the only effective lines of dealing with the problem. While the introduction of the two remedies, antrypol and antrycide, has registered a definite advance in combating equine surra in India, the position in regard to the treatment of advanced cases of the disease remains substantially the same today. Referring to *T. equiperdum*, Schesche (1917) categorically declares that no curative exists for this disease.

The position described by Griffiths (1920) in the Nyasaland Protectorate concerning prospects of obtaining recovery in cases of bovine trypanosomiasis remains almost the same in all countries, including India, to this date. He observes that. in what may be called the 'pre-cerebrospinal stage' of trypanosomiasis, drug intervention combined with proper feeding may bring the affected animal to a state when it can be slaughtered for food or even used for work. In the advanced stage of the disease, the animal is either unable to withstand treatment or entirely refractory to it.

Some reduction in the incidence of the disease might have been expected if infected flies became sterilized after sucking blood from animals recently treated with trypanocidal drugs, but Kleine and Fischer (1923) in Africa have shown that drugs, such as 'Bayer 205' (suramin) have no effect on trypanosomes in the gut of *Glossina*.

The discovery by Fiennes (1950 *b*) of a cryptic stage of trypanosomes in infected animals adds to the gloominess of the picture. This worker claims to have obtained conclusive evidence of the occurrence of these elusive forms in smears of prescapular lymphatic glands of animals that have been prophylactically treated with antrycide against *T. congolense* and *T. vivax* infections. He states that on rare occasions small numbers of these cryptic parasites may be detected in the circulating blood, although it is probable that their periodic appearance is due to an over-spill from an active tissue focus. Fiennes, therefore, distinguishes two stages in *T. congolense* infection of cattle: a primary stage representing all the commonly seen clinical symptoms of the disease, from the incubation to the post-critical period; and a secondary stage characterized by the occasional appearance of small numbers of cryptic parasites in the blood circulation and a reduction of the haemoglobin content of the erythrocytes. The disease, however, continues in an insidious form in the secondary stage and may eventually give rise to severe symptoms resulting in death. While, according to Fiennes, the acute primary stage is often curable, no treatment is known for the secondary stage. He observes : 'Drug prophylaxis and unsuccessful drug therapy tend to inhibit the primary stage of the disease, but permit the development of the secondary stage.' He (1951) further states that relapses may occur after about a year's apparent recovery, with the appearance of drug-fast trypanosomes; or death may occur without the trypanosomes being present in the circulating blood. Fiennes, however, does not make any reference to the state of the cerebrospinal fluid as an aid to prognosis.

Indirect evidence of the limited value of drug intervention as a means of combating trypanosomiasis is provided by the fact that, in spite of systematic campaigns against the disease by means of chemotherapeutic remedies in certain selected and endemic localities in Africa, no really substantial reduction has been effected in the incidence of the disease, although it may be assumed that this has been due—at any rate, partially—to failure in applying the remedies in the early stages of infection. According to figures given by Friedheim (1949*a*) concerning the incidence of the disease in French West Africa alone, the Sleeping Sickness Service examined 4·6 million people in 1946 out of a population of 16 million and new cases of sleeping sickness were detected by them in as many as 50,000, of whom 60 per cent were in the second stage.

In India, surra at the present time has assumed a position of more importance than it did some two decades ago. This, to all appearance, is due not to the spread of this disease during recent years but to the previous absence of reliable data concerning its incidence. Fletcher and Senior-White (1921), dealing with the subject of vectors of surra, has published a map indicating the occurrence of the disease in different localities in India. According to Basu (1945), surra occurs throughout the whole of this country. He gives some figures to show that, out of a total of 12,000 cases of surra registered during 1940-42, 1,000 were in bovines, 6,000 in equines and the rest in camels. Greater importance is now also attached to surra on account of the fact that *T. evansi*, which at one time used to be regarded as more or less innocuous for bovines, has, for reasons not yet determined, been frequently found to assume a virulent form for these animals. Numerous fatal cases of bovine surra have been recorded from Hyderabad-Deccan, Madhya Pradesh, and Madras, while Mudaliar (1944) has discussed some of the problems connected with cattle surra in the last named province.

The high incidence of camel trypanosomiasis in Sudan is indicated by the fact that Bennett (1936*a*), working in that locality, issued 10,081 doses of suramin in 1935 for treatment of the disease, as against 4,219 doses issued by him in 1934. The position in regard to nagana* is thus described in an editorial article in *Nature* (**163**, 78, Jan. 1949): 'Summing up, trypanosomiasis of livestock is probably of a much more serious consequence to mankind than trypanosomiasis of man himself. The fight against both these forms of the infection is waged on two basal fronts, against the transmitting agent, the tsetse fly on

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*As frequent references to nagana occur in this monograph, it would be of interest to indicate here the precise meaning of this term. As pointed out by Curson (1928), the term 'nagana' is applied to the disease caused by *T. congolense*, *T. vivax* and *T. brucei*. He considers this term as being related to the Zulu word 'ku naga', meaning 'to be anxious about,' for the affected animals often walk at a very slow pace—apparently due to weakness—and have thus the appearance of deliberating over something. The condition is called 'unagana' by the local people and 'nagana' is a corrupt form of this word, as used by Europeans. Du Toit (1936) has given the following useful particulars concerning the status of these three species : *T. congolense* is highly pathogenic for cattle, sheep and pigs ; *T. vivax* commonly occurs in cattle but very infrequent in horses ; *T. brucei*, which is of low virulence, occurs in cattle, horses and dogs.

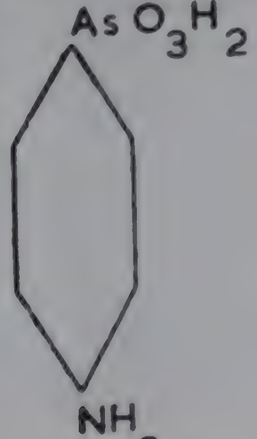
the one hand, and against the actual parasite, the trypanosome, on the other. Potentially, one of the most important weapons in the campaign aimed directly at the trypanosome is chemotherapy; but hitherto this has been directed to a highly useful degree only against human, and not against animal, trypanosomiasis'. The above remark also applies to the conditions obtaining in India, where, however, the fortunate absence of human trypanosomiasis should make it possible to intensify combative efforts against the only species of pathogenic trypanosome, namely, *T. evansi*. As suggested by Ford, Whiteside and Culwick (1948), it is only by a concerted '3-pronged' attack that trypanosomiasis could be dealt with with any substantial measure of success, viz. by the destruction of the trypanosome, the extermination of the vector and the ensuring of general ecological conditions designed to make the entire environment inimical to the continuance of the disease.

A clearer understanding of the precise nature of trypanosomiasis syndrome itself is obviously the prerequisite to the formulation of specific treatment against the disease. As pointed out by Fusco (1910), a substantial advance would be made in devising combative measures if some indications were obtainable concerning the habitat of the parasites during the latency of the disease. He found that, in an infected white rat killed in one hour by arsenophenylglycin, the parasites were present in the liver, lungs, kidneys and heart blood and a small number in the spleen, but none in the brain, spinal cord, bone marrow and lymph glands. No trypanosomes were found in any part of the tissues of a rat killed 24 hours after receiving a curative dose of the drug. In another infected rat killed 24 hours after 1 cgm. atoxyl, no trypanosomes were present in organ smears; subinoculations from heart blood, spleen, liver and lymphatic glands gave negative results. The infectivity of all the organs from animals killed 24 to 48 hours after treatment with arsenious acid was tested by inoculation into rats. In two instances, material from spleen and lymphatic glands alone and in a third from spleen alone were infective, although no trypanosomes were found in any of these. When killed more than 48 hours after drug inoculation, the other organs—liver, lungs, kidneys, etc.—proved infective. Concerning the latency of trypanosomiasis referred to by this worker, it is of interest that Walker (1912) in India claimed to have observed the schizogony of *T. evansi* taking place in the spleen of the invertebrate host and he refers to its possible relationship to the latency of the disease. Dealing with the subject of pathology and therapeutics of nagana, Hornby (1937) mentions the existence of only fragmentary information even on such fundamental points as (*a*) the location of the parasites at different points in the tissues of the host at different stages of the infection; (*b*) the manner in which a species of trypanosome becomes pathogenic for its host; (*c*) the nature of immunity induced by infection; and (*d*) the factors responsible for different results being produced under identical conditions as regards treatment, test animals and strains of trypanosomes. He deplores the fact that 'most writers of text-books on animal diseases ignore altogether the pathology of animal trypanosomiasis'.

A striking example of the divergence of views over a fundamental problem of trypanosomiasis relates to the question of the virulence of a strain with its drug-fastness. As pointed out by Brauwere (1947), it is held by certain workers that arsenic-resistant strains are of feeble virulence, while according to others, such strains may at times cause severe outbreak of the disease.

Except for the occurrence of a disturbance of the sugar metabolism, little substantial information is on record concerning biochemical changes in the advanced stages of trypanosomiasis. Discussing the subject of pathology and immunity in cattle trypanosomiasis, Fiennes (1950*a*) states that there is no evidence to show that death in acute trypanosomiasis is due to a trypanotoxin, but that it is possibly due to anaphylaxis. Concerning the relapsing properties of trypanosomes, he ascribes these to antigenic variations. He uses the expression 'aggressive properties' of the parasite and considers the subject under the headings of reactive and non-reactive hosts; adaptability; serum and drug-fastness; and virulence. According to Oye and Peel (1951), sleeping sickness in its final stage assumes the form of chronic meningo-cephalitis, with large quantities of lymphocytes and plasmocytes in the perivascular infiltrations. Of these two cellular elements, the lymphocytes—some of which show signs of degeneration—are accredited by them with performing the function of secreting antibodies, while the plasmocytes absorb and accumulate lipins, which are produced as a result of defective metabolism of the cerebral cells infected with the parasites. These accumulations eventually take the form of large conglomerations of bladder-like deposits and are characteristic of the disease, being unlike the vacuolated plasmocytes which one sees in other organs and diseases.

The possibility of any special class of food influencing the course of trypanosomiasis obviously requires to be eliminated before conclusions can be drawn as to the precise nature of the syndrome of the disease. French and Hornby (1934, 1938) have thrown some light on this point from their studies on *T. congolense* infection in cattle. According to them, nutrition higher than that of maintenance requirements has no effect on the course of the disease. They further observed that nutrition resulting from basal hay ration with certain food supplements had also no effect on the disease. In a second series of experiments, they first sterilized the circulation of infected animals with antimosan or tartar emetic and then fed one group of these animals on cotton seed cake and another on ferrous sulphate and copper sulphate in addition. Little difference was observed in their weights taken periodically, showing that it was the plane of nutrition and not blood-improving drugs that operated in bringing about recovery. These observations will obviously require to be repeated, under varying conditions of food and animal breed, before definite deductions can be made. In the present state of our knowledge, the treatment of trypanosomiasis will, therefore, require to be continued on quasi-empirical lines, making what use is possible of the extensive findings that are on record on the trypanocidal properties of drugs. One of the striking discoveries in this field is that of the existence, in certain classes of drugs, of an intimate relationship of their chemical constitution to their trypanocidal value. In an illuminating paper, Tréfouel (1938) emphasizes the desirability of first choosing the chemical group ('domaine') that might appear to hold out promise of yielding an effective remedy for the form of trypanosomiasis in question. When some indication has been obtained as to the general chemical character of the drugs required, one might embark upon an investigation into the possibility of producing more strongly trypanocidal agents by processes of synthesis. Tréfouel refers to the fact that, as first shown by Ehrlich and later by Fourneau and others, the trypanocidal properties of certain chemical bodies are governed by a predetermined limit ('terme'), so that the slightest alteration in the chemical configuration of these bodies results in the elimination ('bouleversement') of these properties. A classical example of such a drug is provided by *p*-aminophenylarsenic acid (atoxyl-tryponyl):



Ehrlich, after establishing the exact formula of this drug, succeeded, through a series of modifications of its constitution, in causing variations in its toxic properties and later in demonstrating that, by means of such modifications, the effect of certain arsenicals might be diminished or exalted and even extended to an altogether new group of diseases, namely, to that of spirochaetoses. In illustration, Tréfouel cites the example of 'atoxyl-tryponyl' mentioned above. He points out that the action of phynylarsenic acid is profoundly altered not only by introduction of certain groups in the molecules NH_2, OH, etc., but also by the relative position of these groups. Thus, phenylarsenic acid is toxic for the mouse at 1 mg. and is non-trypanocidal. On the other hand, *p*-aminophenylarsenic acid is more toxic only at 3 mgm., and at this dosage it may cure 30 per cent of mice. It would thus appear that it is the amino group NH_2 that matters in determining the toxicity of the molecule. Furthei, ortho-and meta-aminophenylarsenic acids are much less active, so that the para position is the optimal ('la position optima du substituent unique NH_2 est la position para'). The case is, however, very different with oxyphenylarsenic acid; here the *m*-oxyphenylarsenic acid is the more active. The existence of a relationship between the chemical constitution and the trypanocidal and neurotoxic actions of aromatic arsenic acids has similarly been demonstrated by Friedheim (1938). Instances of intimate relationship between the chemical constitution of a drug and its trypanocidal properties are also provided by some of the other arsenicals and also by antimonials. As shown by Yorke and Murgatroyd (1935) and others, pentavalent aromatic compounds of arsenic and antimony are only slightly trypanocidal, whereas in their trivalent form, they, as also non-aromatic trivalent compounds, are highly trypanocidal. These authors further observed that, while trivalent arsenicals speedily sterilized the peripheral circulation, they were of little curative value for infected rabbits.* On the other hand, the corresponding pentavalent compounds, although slow in their sterilizing action, produced cure in a large number of cases. According to them, the reduction of certain pentavalent compounds to the trivalent form is brought about by the action of haemoglobin of the red blood-corpuscles. Incidentally, Yorke and Murgatroyd refer to the fact that the chemical side of chemotharapy has far outrun the biological side, this, according to them, being due to the fact that chemotherapeutic investigations have taken two separate directions—one by chemists and the other by biologists.

THE IDENTITY OF TRYPANOSOMES

It is almost needless to stress the necessity of carefully determining the species of a trypanosome before undertaking experiments for testing the trypanocidal power of a drug against it, for the species may be one not previously recorded, so that erroneous conclusions may be arrived at on account of the assumption that the particular parasite does not occur in the locality concerned. A case in point is the finding, made in India by Stirling (1921), of what he regarded as *T. congolense*, although the finding has not been confirmed. Mudaliar (1945), also in India, records a small species of trypanosome from the buffalo measuring 21 $\mu \times 1 \cdot 25$ μ and designated by him as *T. evansi* var. *ravi*. He found this species more pathogenic for rabbits than for guinea-pigs. It is possible that the species recorded by Stirling and Mudaliar are only polymorphic forms of *T. evansi*. This possibility is suggested by the fact that Hoare (1952) found that, in a laboratory strain of *T. evansi* from camels, the proportion of short forms varied from nil to 61 per cent and this led him to conclude that it was only the inconstancy of polymorphism that constituted the difference between *T. evansi* and *T. brucci*. As a matter of fact, the precise systematic position of even some of the common species of trypanosomes is still disputed. Thus, Kleine (1928) adduces reasons to show that *T. gambiense* is identical with *T. rhodesiense*, but according to him, the latter is different from *T. brucei*. On the other hand, Yorke, Adams and Murgatroyd (1930) showed that, while human serum was lethal for *T. equiperdum*, *T. rhodesiense* and *T. congolense*, it had no action on *T. gambiense* and, according to them, this explained why man was susceptible to *T. gambiense* and immune to pathogenic trypanosomes of animals. They consider that both *T. gambiense* and *T. rhodesiense* are identical with *T. brucei*, that is, both of them originated from *T. brucei*, the pronounced pathogenicity of *T. gambiense* for man being due to numerous passages through man. The view advanced by Yorke and his collaborators is also shared by Mesnil (1930). It is noteworthy that *T. congolense*, the status of which has seldom been disputed, is regarded by Bevan (1937) as synonymous with *T. pecorum*.

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*Possibly, this is to be largely attributed to chronicity of the disease in these animals.

The species of host from which it has been collected might, in certain instances, provide a surer indication of the specific identity of a trypanosome than its size. Manresa and Gonzalez (1936) found that trypanosomes from cattle, when injected into rats and horses, rapidly increased in size, the measurements according to the host being as follows: cows, 18·3μ; baffaloes, 19·8μ; rats, 25·1μ; horses, 27·3μ.

The possibility has also to be borne in mind that a species of trypanosome known to be innocuous may, for reasons not determined, assume a virulent form. As has already been stated, *T. evansi*, which in India was at one time regarded to be generally harmless for cattle is now known to cause serious outbreaks of surra amongst these animals. Wyssmann (1935) records 2 cases of fatal *T. theileri* infection in cows, while Statineau *et al.* (1936) mention a severe outbreak of *T. lewisi* infection in experimental white rats. According to them, *T. lewisi* may 'acquerer des epizooties séverés'. Bourgeois (1941) records the occurrence of *T. theileri* in the uterus, spleen and liver of a cow that died two weeks after calving.

The varying degrees of resistance to drugs exhibited by different strains of the same species of trypanosome sometimes introduces a disturbing element in the progress of studies on their chemotherapeutic value. The precise factors responsible for such variation do not appear to be understood but a few examples may be cited as illustrative of the circumstances in which variation has been found to occur.

Teichmann (1917) found that different strains of *T. brucei* within the limits of East Africa alone showed marked difference in their sensitiveness towards arsacetin. Borchgrave (1939) found that an arsenic-resistant strain of *T. gambiense* was much more virulent than an ordinary strain of this species. Hawking (1940) records the observation that an old strain of *T. rhodesiense*, maintained in laboratory, responded more readily to tryparsamide that had penetrated into the cerebrospinal fluid than local strains of the same species. He also found that the occurrence of trypanosomal lesions of the cerebral membranes had no 'constant influence' on such penetration. The reaction of an infective dose of trypanosomes may be in some cases, as pointed out by Kligler and Rabinowitch (1927), due to the condition of the animal concerned. The bulk of available evidence points to the conclusion that the virulence is a property of the strain and also that it is not governed by the site at which the parasites are inoculated. They further observe that, for experiments of this kind, the rat was a 'more satisfactory gauge' than the guinea-pig, because in the former the disease quickly progresses to a fatal issue. Zschucke (1936) found that the results of inoculating a strain into the circulation did not differ from those of inoculating it into the cerebrospinal system, so that the route had little or no relationship with the virulence of the strain ('nennenswerte Unterschiede im Krankheitverlauf nicht beobachtat').

A numbr of observations are on record to show that the same strain of trypanosomes, after numerous passages over a long period in the laboratory, may undergo considerable variation in virulence. Kunert (1936) records such variation in *T. brucei* and refers to similar observations made by Schilling, Duke and others. He considers that such variations may sometimes be responsible for the apparent difference in the effectiveness of a drug when tested repeatedly on the same strain in the laboratory. Murgatroyd and Cossor (1937) also observed that a strain of *T. brucei*, during five years' maintenance in the laboratory, suffered a decrease in its pathogenicity for guinea-pigs and mice, while it ceased to be resistant to arsenicals. On the other hand, Corson (1939) found that *T. rhodesiense* retained its infectivity for man even after more than four years of passage through sheep and goats, so that it is possible that the degree of virulence of a strain is determined by factors other than its continuous occurrence in a particular class of hosts.

The infectivity of a strain is also known to be affected by the action of the kind of drug which has been used against it. Thus, Dubois and Kohn (1939) found that, when infected mice were treated with neosalvarsan, novoflavine or suramin, the trypanosomes from them were non-infective but not when they were treated with tryparsamide or tartar emetic.

The occurrence of such variation in virulence of a strain minimizes the value of cross-immunization tests as a means of differentiating closely related species; for, as remarked in the course of an editorial comment in *Sleeping Sickness Bulletin* (**1**, 380; 1909), 'the fact that animals immune to one strain of nagana promptly succumbed to a strain which differed only in that it had been kept in a different species of animal shows the untrustworthiness of cross-immunization experiments for the distinction of species. It also gives a hint of the probable way in which species of trypanosomes pathogenic to mammals were formed'.

THE STANDARDIZATION OF DRUGS AND OF CHEMOTHERAPEUTIC TESTS

It is unfortunate that methods for testing the trypanocidal efficacy of drugs have not been standardized. The results recorded by different workers in this field can, therefore, hardly be regarded as comparable.

In standardizing drugs, a serious complication will have to be reckoned with if, as found by Breinl and Nierenstein (1909) in the case of arsenophenylglycin, the possibility of cure diminishes as the weight of the animal increases. These workers report that in *T. brucei* infection they obtained satisfactory results when the experimental animals were guinea-pigs and middle-sized dogs, but donkeys proved resistant to treatment. According to Massaglia (1909), the natural defence against this form of infection is not due to the action of leucocytes (phagocytosis) but to specific antibodies. For determining the standard doses of drugs, Raven (1910) considers it a good method to increase the dose rapidly till one is reached which consistently produces toxic symptoms; for, it entails much expenditure of time if the dose is gradually raised until one is found to produce the largest number of cures. Kolmer *et al.* (1917*a*) recommend the following three broad principles for standardizing drugs against *T. equiperdum* infection in white rats: (1) to study under microscope the degree of mortility exhibited by the parasites after exposure to suitable dilution of the drug; (2) to test the viability of the parasites after exposure to the action of the drug, then washing, centrifugation and intraperitoneal injection into rats; (3) exposing a mixture of equal parts of the drug and infected blood to a temperature of 37°C. to 40°C. and then injecting the mixture into animals. This last method is called by them 'in-vitro-vivo method'. In another paper, the same authors (1917*b*) mention the existence of a numeric relationship of infection to chemotherapy of experimental trypanosomiasis, i.e. relationship between the number of trypanosomes injected and the degree of curative action exhibited by the drug. Thus, while rats infected with 500,000 *T. equiperdum* trypanosomes were sterilized on being injected with 0·101 gm. of arsenobenzol per 100 gm. of rat, sterilization was not obtained when the number of parasites injected exceeded 2,000,000, although in this case the appearance of the parasites in the peripheral circulation was suppressed. On the other hand, Kligler and Rabinowitch (1927) found that the duration of the disease increased as the number of parasites injected was reduced. Barlowatz (1933) concluded from his expriments that the toxicity of an arsenical for small rodents and for man might not be parallel; it may be inoffensive for animals and noxious ('nouif') for man. Collier and Seegert (1933) had already arrived at similar conclusions from their observations on the efficacy of myosalvarsan, fouadin and suramin for nagana in mice and guinea-pigs. They also observed the toxicity of the same drug differing with a different host.

It is desirable to determine the chemotherapeutic efficacy of a drug by actual tests *in vivo*, for it has been conclusively shown that no generalization can be made as to the relationship between the results obtained *in vitro* and those obtained *in vivo*. Thus, as pointed out by Yorke and Murgatroyd (1935), while many compounds are active both in *in vitro* and *in vivo*, suramin although inactive *in vitro*, shows *in vivo* an activity analogous to that of trivalent compounds. For standardizing suramin in regard to its toxicity, Van den Branden (1938*a*) suggests the subcutaneous injection of a fixed quantity of a fixed concentration of the drug, so that a constant number of animals (mice) is alive and well after a definite period. In a later paper, he (1940) suggests the slow injection by the intravenous route of a 5 per cent solution of the drug in doubly distilled water, in a dose of 0·40 gm. per kilo, to 5 mice each weighing 20 gm.; not more than two of these mice should be dead within six days. Van den Branden (1941) also tested the possibility of quickly estimating the efficacy of neo-arsphenamine by the action of its fumes on white rats infected with *T. brucei*; the method did not prove successful. He says that he does not recommend ('préconiser') this method but prefers what he calls the method of Kolle and Leupold. Kraneveld and Umboh (1939) observed that, under identical conditions as regards body weight, age, etc., the experimental animals sometimes reacted differently to the same drug, so that a large number of trials required to be carried out with a drug before conclusion could be drawn as regards its efficacy. The same authors found that the number of cures varied inversely with the number of fractions in which a dose was split up.

Fulton and Yorke (1943*a*) use the expression 'minimum effective dose' (M.E.D.) as being equivalent to the minimum dose sufficient for the purpose of sterilizing the blood of at least 80 per cent of the treated mice, irrespective of the time. The expression 'minimum curative dose' (M.C.D.) is used by them for the minimum dose sufficient for the purpose of curing in 30 days. As is well known, the usual method of determining the chemotherapeutic index—in other words, the efficiency—of a drug is to divide the maximum tolerated dose by the minimum curative dose: C.I. = M.T.D./M.C.D. The objection made by certain workers against this method is that the index thus determined may vary with the species of the host and the route of administration (*cf.* however, Zschucke, *ante*). The method for determining the chemotherapeutic index, as recommended by Trevan (1927) and Wien (1946), is based on the ratio of the median lethal dose to the median effective dose:

$$\text{C I.} = \frac{\text{Dose which kills 50 per cent of animals}}{\text{Dose which cures 50 per cent of animals}} = \frac{\text{LD50}}{\text{CD50}}$$

A number of useful suggestions have been made on the subject of standardization of drugs in the report of the third session of the International Scientific Committee for Trypanosomiasis Research held in June 1951. Although these suggestions specifically concern melarsen and its compounds, they are of sufficient general interest to merit inclusion here. The Committee have stressed *inter alia* (1) the imperative necessity of conducting tests for each drug in respect of its toxicity and remedial properties before it is sent out for use under field conditions; and (2) the adoption of measures for making available all laboratory protocols for the inspection of workers. In the case of diamidines—and this should apply to several other drugs—they recommend expressing the dosages in terms of base instead of salt.

The standardization of prophylactic doses of drugs necessitates more extensive trials inasmuch as the duration of the protection period for different doses has to be ascertained separately. In the case of pentamidine, however, Launoy and Logodsky (1946*a*) found that the size of the dose varied directly ('proportionnelle') with the duration of protection. Possibly, this may be found to hold good with certain other drugs.

THE DETERMINATION OF DOSES

The two main principles of chemotherapeutic treatment, as succinctly stated in *Sleeping Sickness Bulletin* (1, 35; 1908), are: (1) Combined therapy and (2) alternation of trypanocidal agents. To these may be added what is known as (3) *therapia sterilisans magna*. The first of these has been explained as follows: 'The combinatory method has the advantage that each drug can be used in doses much under those which are poisonous to the organisms; if we attack the enemy from different sides simultaneously the forces can be divided'. Concerning the second, it is stated: 'We must ring the changes on the drug which are at our disposal. A trypanocide which does not at once sterilize the tissues loses its effect if the administration is persevered with. There is produced an habituation of the parasites to the remedy. This event should be forestalled by changing the point of attack, by bringing into play other drugs which combine with other portions of the protoplasm of the parasites, as Ehrlich has indicated. It may be that by a timely change of remedy we can bring fresh forces to bear on parasites weakened by the first attack and therefore less able to resist a second'. The third principle enjoins the administration of a drug at the maximum tolerated dose so as to preclude the production of resistant strains. Uhlenhuth and Woithe (1908) tersely define this principle as " 'as early as possible, as much as possible'. If large doses had been given at first the result might have been different". The application of this principle evidently involves a certain amount of risk in view of the possibility of less resistant individuals developing toxic symptoms on receiving a heavy dose of the drug.

Tsuzuki (1911) has suggested some further improvement on treatment formulated on the principle of *therapia sterilisans magna*. Referring to the treatment of nagana in mice, he observes: 'In the treatment of experimental nagana, the combination of several therapeutically active substances acts better and produces a surer curative effect in respect of *therapia sterilisans magna* than do larger doses of the undivided components'. One further principle laid down by him is that the combination of remedies from the same chemical group is less effective than that from different but closely related groups.

Ciuca (1914*a*) suggests the unique method of increasing the efficacy of atoxyl against *T.evansi* and *T.brucei* infections in rabbits by injecting 1·5 to 2 c.c. of a 1 per cent solution of terebene into the thigh of these animals. The injection is followed by abscess formation in 4 or 5 days. It should be opened and the terebene-smelling pus drained out. The abscess is then dressed and atoxyl injected as usual. Ciuca considers it probable that the injection of terebene facilitates the formation of trypanotoxyl. For inexplicable reasons, the practice of undertaking investigations into the possibility of devising methods designed to mitigate the physical pain—due to allergy, etc.—following the injection of certain classes of drugs appears to have ceased altogether. The usefulness of this line of work is illustrated by the fact that Thiroux (1909*a*) forestalled the untoward symptoms of intravenous administration of emetic by injecting 20 cgm. caffeine subcutaneously earlier, although the injection was 'un leger inconvénient', for it produced vaso-constriction 'assez accentuee'

The question of selecting suitable types of laboratory animals for studying the progress of trypanosomiasis or testing the efficacy of a drug is of greater importance than appears to be generally realized. Nierenstein (1912) expresses doubt as to the suitability of mice owing to their tolerance for a number of drugs. Guinea-pigs, according to him, show better the fluctuations of temperature and also the result of treatment, although they may often die without showing parasites in the circulating blood. He further states that rabbits are best suited for studying the chronicity of the disease. Pearce *et al.* (1918) have shown that mice and rats are suitable test animals when quick results are desired, but the syndrome of trypanosomiasis in rabbits assumes a chronic character (*cf.* Nierenstein, *ante*) and therefore presents a closer similarity to natural infection, so that the two types supplement each other. They point out that, in records of work on trypanosomiasis, one hardly come across 'any clear-cut application or even recognition of these principles'. Similar views have also been expressed by Pearce and Brown (1919) in connection with their studies on the efficacy of tryparsamide.

Routes for Administration of Drugs

For the oral administration of durgs, Marks (1909) suggests the intrastomachal method ('intrastomacher Zuführung der Heilstoffe'), such as by means of a sound, in order to enable large quantities of the drug to be introduced into the stomach. He records having produced cure in mice by a single dose when used in this manner and points out that, if the drug is administered in cakes, its quantity and absorption remain uncertain. The oral method would appear to present distinct advantages in some cases over the parenteral methods. Thus, Levaditi *et al.* (1926) found tryparsamide and certain other arsenicals highly effective prophylactically when administered by the mouth against nagana in rabbits. On the other hand, Zschucke (1936), referring to suramin states that he found the drug unsatisfactory when administered by the oral route, this being attributed by him to the different degrees of absorption in different individuals.

Bevan (1926) claims to have caused temporary sterilization of the circulating blood of guinea-pigs infected with *T.rhodesiense* and *T.brucei* by dipping them several times in arsenical solutions. Cooper's dip used at a strength of 1:300 was ineffective, but the disappearance of the parasites took place when the animals were given 3 to 5 dippings in a 1:156 strength of the dip. Bevan attributes this effect to the gradual absorption and accumulation of arsenic in the tissues of the animals. He, however, considers the experiment to have been 'inconclusive owing to the scarcity at any time of trypanosomes in the blood even in the control animals'.

It is of interest that even the possibility of obtaining cure of trypanosomiasis through inhalation of drugs has not been left untested. Thus, Silberschmidt (1935) enclosed *evansi*-infected mice and rats into a box sprayed with a solution of tartar emetic. The enclosed animals lived for more than 30 days and the controls for 3 to 9 days. Van den Branden (1938*b*) also obtained temporary sterilization in 14 *brucei*-infected rats by subjecting them to the action of the vapour of suramin.

Fulton and Yorke (1943*b*), in their studies on *T. rhodesiense* infection in mice, found that the maximum tolerated dose of novarsenobillon and tryparsamide was the highest by the subcutaneous and the smallest by the intravenous route, but that both tryparsamide and reduced tryparsamide gave equally good results by the intravenous and intraperitoneal routes. They obtained the 'best therapeutic indices by subcutaneous administration'. Variations in efficacy were also observed by them in accordance with the species of parasite against which treatment was applied. Thus, in *T. rhodesiense* infections, 4:4-diamidino monomethyl stilbene, when given subcutaneously, showed the highest therapeutic index, while in *T.congolense* infections, the subcutaneous administration of this drug was not more effective than that of 7 amino-9 (*p*-aminophenyl)—10 methyl phenanthidinium chloride. On the other hand, the intraperitoneal administration of the stilbene in *T. congolense* infection gave better results than that of the phenanthridinium compound.

It may, however, be assumed that there is scope for improvement in the actual technique of administration by any particular route. Thus, in the report of the Chief Veterinary Officer, Rhodesia, for 1923-24, the posterior auricular vein, rather than the jugular vein, is recommended as a suitable site for the injection of tartar emetic, although the reason for this recommendation is not clear. Fowler (1945) in Gold Coast worked out a method for the administration of massive doses of tryparsamide by the intravenous route. The patient was made to lie down and, with a special apparatus designed for the purpose, the drug was injected at the rate of 40 drops a minute, as much as 2 pints of the solution being thus injected in 8 hours. The actual amount of tryparsamide injected each day was sometimes as much as 2 gm, the treatment being continued for 6 to 9 days with a day's interval when considered necessary. Fowler treated a total of 35 cases of sleeping sickness by this method and the results are reported by him to have been highly encouraging.

A natural corollary of the failure of drugs, when introduced into the blood circulation, to exert their action on the trypanosomes lodged in the cerebrospinal system has been the development of the intrathecal method of injection designed to reach the parasites at this otherwise inaccessible site of infection. As early as 1909, Balfour suggested the injection of a trypanocidal drug into the blood and, on the patient showing signs of improvement, the injection of his own serum into the cerebrospinal fluid. Shircore (1921) cites Thinoux and D' Aufreville as also having suggested the intrathecal method of injection of serum about the same time as Balfour. Reichenow (1914) records having given intraspinal injection of neosalvarsan successfully. Concerning the possibility of untoward results being produced when administered in this manner, Kopke (1916) says that the method entails no danger, although the degree of benefit derived from it is uncertain.

Marshall (1920, 1921*a*, 1921*b*), Marshall and Vassallo (1921) and Eyre and Marshall (1921) made use of this method with encouraging results in the treatment of sleeping sickness. The last named workers injected intraspinally neokharsivanized serum in combination with the intravenous injection of the drug. According to Marshall, the non-penetrability of metallic drugs into the cerebrospinal fluid is due to a pathological change in the meninges caused by the trypanosomes that have found their way into the central nervous system, implying thereby that normally such drugs are capable of penetrating that location. Marshall (1921*a*) gives a sketch of appliances used by him for intrathecal injection. Yorke (1921) published an informative review of Marshall and Vassallo's work and portions of this review will bear quotation : 'It is doubtful, however, if Marshall and Vassallo are correct when they state that the fact that the metallic medicaments do not reach the cerebrospinal fluid is due to a pathological change in the meninges brought about by the trypanosomes in the central nervous system. Halliburton (1921) pointed out at a meeting of the Royal Society of Tropical Medicine and Hygiene in the discussion which followed Marshall's paper, that the cerebrospinal fluid is a secretion and that both in health and disease the choroidal cells maintain their rôle of a stalwart barrier, keeping back foregin materials circulating in the blood. Only one or two volatile materials, e.g. alcohol get through and there is no passage of poisonous materials like arsenic or antimony from the blood to the cerebrospinal fluid. Prof. Carl Voegtlin, on the contrary, informed the reviewer in a private communication that he had frequently found traces of arsenic in the cerebrospinal fluid of normal rabbits and syphilitic patients after intravenous injection of large doses of salvarsan and neosalvarsan; and, further, that he had observed that intravenous injection of large doses of these drugs and of atoxyl caused within 24 hours a great diminution in the number of parasites in the cerebrospinal fluid of rabbits which he had subdurally injected with a heavy suspension of '*T. equiperdum*.' Yorke considers that the possible explanation of the discrepancy between the two views is that arsenic and antimony may penetrate into the cerebrospinal fluid only when they are injected in large quantities in the circulating blood. Concerning the related question whether relapse strains of parasites in the circulating blood originate from the cerebrospinal fluid. Yorke expresses the view, supported by experimental evidence, that this may be true for advanced stages of the disease but that, in cases of relapse in early stages of the disease, the strains originate from tissue spaces (e.g. cornea) and circulation backwashes, where the trypanosomes remain protected from the action of drugs. Voegtlin *et al.*(1923); investigated the relative penetrability into the cerebrospinal fluid of arsphenamine, neoarsphenamine, silver arsphenamine and sulphur phenamine,when these drugs were injected intravenously. The penetrability was determined by studying (1) the disappearance of arsenic from the circulation and by the arsenic content of the cerebrospinal fluid and the brain tissue; (2) their effect on the parasites injected into the cerebral subarachnoid space. The second method was found to be more effective. The result of their investigation evidently confirms the findings of Voegtlin referred to by Yorke (1921, *ante*).

Lefrou and Ouzilleau (1922*a*) altogether discredit the therapeutic value of intrathecal injection of medicated serum advocated by Marshall and Vassallo and they also mention having found the direct intrathecal injection of drugs as producing fatal results. They conclude from their work with atoxyl and neosalvarsan that the intrathecal injection does not present any superiority over the intravenous. Van den Branden and Van Hoof (1922*a*) consider it likely that Marshall and Vassallo would have found the drugs equally effective when injected by the intravenous route alone. They mention having treated 12 cases of sleeping sickness by the intraspinal injection of salvarsanized serum. Ten of these cases relapsed and the other two worsened, although they did not relapse. Van den Branden (1922 *a*), referring to Marshall's method, says that he tested it on 18 cases of sleeping sickness and found it of little value. Marshall and Vassallo (1923) in a rejoinder quote figures to prove the success of the method, namely, an intravenous injection of salvarsan, followed 24 hours later, by an intrathecal injection of salvarsanized serum. They consider it possible that 'Yorke, like many others in the present year of grace, has a personal aversion to the local treatment of infections of the central nervous system'. They claim to have obtained recovery in 66·6 per cent of the cases treated by them and in another instance in 94-100 per cent of the cases. The controversy on this subject would appear to have come to a close with Marshall and Vassallo's (1926) own admission that the intrathecal method yielded little success when tested on cases in the Nile district.

Suramin proved in the hands of Mayer (1921) to be a drug suitable for intrathecal injection in the treatment of experimental dourine. He records having successfully treated by this method a guinea-pig, and a dog weighing 14,000 gm., at a dose of 0·02 gm. and 0·7 gm. respectively. In India, Edwards (1926) employed the intravenous-intrathecal method of injection for the routine treatment of equine surra. The drug was highly diluted, the dosages being 1/500th of that used for intravenous injection. The dosages for the two routes were worked out on the basis of the relative weights of the cerebrospinal tissues and the blood volume. The method has been fully described by him with illustrations. Saunders (1928), however, records having obtained cure only in one out of five human patients treated by the intravenous-intrathecal injection of suramin in small dilute doses. The injection was found by him to produce alarming symptoms.

Shircore (1928) and Peruzzi (1928) view all forms of intraspinal injection with disfavour on account of the likelihood of uninfected cerebrospinal fluid acquiring trypanosomes from the blood as a result of such operation.

Radra (1938) suggests the adoption of what he calls 'Schacherl's method' of ensuring the penetration of drugs into the cerebrospinal fluid after these drugs have been introduced into the circulating blood. According to him, the destruction of the meningea barrier can be effected by the injection into the spinal canal of arsebenyl dissolved in 9 c.c. of cerebrospinal fluid and mixed with a small quantity of distilled water. The injection is given slowly and the patient is made to lie down for 24 hours in a horizontal position. After this, he is given the usual course of treatment intravenously with trypanocidal drugs. By this method, he claims to have brought to normal the cerebrospinal fluid in 6 out of 7 patients treated by him. In reviewing Radra's article, Yorke expresses his inability (shared by the present compiler) to understand the mechanism whereby the meningeal barrier is destroyed.

Lourie (1943) has discussed the relation of the so called 'blood brain barrier' and cerebrospinal fluid with the efficacy of drugs employed in the treatment of sleeping sickness. He gives the probable location of this 'barrier' as the walls of the cerebral capillaries, it being, therefore, necessary for drugs to pass through the capillary endothelium into the perivascular and pericellular spaces. The property of a drug favourable for its passage through the 'barrier' has not been determined with any degree of precision. Suramin which is a highly effective trypanocide when present in the general blood circulation, is known to possess little power of penetration into the cerebrospinal canal, while tryparsamide is generally recognized to be a drug which, although not particularly effective in the circulating blood, quickly finds its way into the cerebrospinal fluid. According to Lourie, the electro-chemical properties constitute a factor that determines the penetrability.

Whatever be the physical relationship between the circulating blood and the cerebrospinal fluid, the immediate desideratum obviously is the search for a drug endowed with the property of readily penetrating into the meninges when it is introduced into the blood. Reichenow (1921) claims to have cured five cases of cerebrospinal infection in sleeping sickness by injecting into the circulating blood urotropine with alcohol, combined with the administration, on two consecutive days, of 0·5 gm. atoxyl and 3 gm. urotropine.

TERMINOLOGICAL INEXACTITUDES IN CHEMOTHERAPY

It would seem open to question whether 'chemotherapy' is an appropriate term to use for designating a form of treatment which, as originally designed by Ehrlich, is to be maximally parasitotropic and minimally organotropic ; for, the term by itself hardly clearly indicates the difference of this form of treatment from that of therapy or pharmacology. Merillat (1938) has suggested the expression 'physio-chemistry' in place of 'chemotherapy', in recognition of the pioneering services rendered in this field by both physiologists and chemists, but such an expression would be more in the nature of a tribute to early workers than as an indication of the scope of this science. 'Parasitotropy' or 'parasitotherapy' might be a more appropriate term for this science. The former, however, as pointed out by Morgenroth and Halberstaedter (1910*a*), does not take into account the sensitiveness of the protozoal cell to the poisonous effect of the drug. They, therefore, consider that the two terms 'parasitergy' and 'organergy' together more correctly express the entire action of a drug. They explain these two terms as follows :

Parasitergy = Parasitotropy × sensitiveness (parasites)

Organergy = Organotropy × sensitiveness (host)

Elucidating further, they cite the example of quinine when used on nagana-infected mice. This drug was found quite ineffective when used subcutaneously, but produced better results when given orally with Albert biscuits. They, therefore, express the action of quinine in this case as follows : When quinine is injected subcutaneously organergy = parasitergy ; when it is fed organergy < parasitergy.

A reference may also be made here to the expression 'prophylactic therapy', as distinct from 'curative therapy', used by Aubert and Heckenroth (1913*a*) to designate measures directed to sterilizing the peripheral circulation so as to render the subject incapable of serving as a reservoir of the disease. To avoid confusion, it would obviously be desirable to restrict 'prophylactic' to the conventional sense of preventing a subject from contracting disease.

The terms 'first stage', 'second stage' and 'third stage' used to express the extent to which trypanosomiasis has progressed in a patient call for greater precision and may even be avoided altogether ; for, as Leger (1931) points out, cerebrospinal involvement may occur in the so called 'first stage' of the disease, so that 'lymphtico-blood', 'meningeal', etc. would be more appropriate expressions to designate stages of the disease. He, however, concedes that the relative duration of these periods is 'plus grande élasticité'. A similar view has also been expressed by Leger in a later (1932) paper published by him in collaboration with Marcel and Sicé. In this paper, the authors observe : 'n'y a pas dans la Maladie dué Sommeil, une systematisation inflexible en deux périodes distinctes'. According to Harding (1945), neither the presence or absence of trypanosomes in the circulating blood nor clinical symptoms can be regarded as a reliable guide for determining the stage of sleeping sickness. It is the picture of the cerebrospinal fluid on which one must depend for correct diagnosis of the stage to which the disease has progressed. This remark should also apply to trypanosomiasis of animals.

A good deal of confusion exists about the nomenclature of certain drugs on account of the different trade names by which they are referred to in chemotherapeutic literature or are advertised for sale. Henry and Gray (1935) have given a useful list of equivalents for some of these drugs. The list is reproduced below, with the addition of some newer names marked with asterisks.

(1) *Tryparsamide*: tryparsone, glyphenarsine, tryponarsyl, trypotan, novatoxyl, tryparsonum*, sodium-N-phenylglycine-amide-*p*-arsonate*, naphuride*.

(2) *Acetylarsan*: Stovarsol.

(3) *Salvarsan*: 606, arsphenamine, neosalvarsan, novarsenobenzol, novarsenobillon, Ehrlich 914.

(4) *Germanin*: Bayer 205, Fourneau 309, moranyl, naganol, suramin*, belganyl, antrypol*.

(5) *Trypaflavine*: 303, acriflavine, gonacrine.

Henry and Gray remark that 'it is a counsel of perfection, which not even the most enthusiastic of organic chemists would urge, to ask medical men to use the systematic chemical names of synthetic drugs'.

Concerning the equivalents of germanin, Hawking (1941) says that suramin is hygroscopic and absorbs moisture which may be as much as 40 per cent, while antrypol has a water content of about 14 per cent. 'Bayer 205' (in ampoules) is stated by him to be anhydrous. These differences in water content are not known to affect their toxic or therapeutic properties.

CHAPTER I

Diagnostic Methods for the Detection of Trypanosomiasis

THE drugs in use in modern chemotherapy of trypanosomiasis usually sell at a price hardly within the reach of the average stock owner in India, so that one has to make certain by the adoption of efficient diagnostic methods that indication for the use of such drugs really exists.

Although the examination of blood smears combined with biological tests constitutes in a majority of cases a fairly efficient method for the detection of trypanosomiasis, it is now well known that this diagnostic method is much less dependable than it was regarded in the early days of trypanosomiasis work—particularly in the so called 'cryptic' infections, or when the infection is not sufficiently generalized, or when the parasites have sheltered themselves in safe retreats, such as in the cerebrospinal canal or in tissue spaces. In such cases, some form of serological test is an obvious necessity.

The more important serological methods at present in use for the diagnosis of trypanosomiasis are: formo-gel, mercuric chloride, complement fixation tests and red-cell adhesion. A number of other diagnostic methods have been brought into use from time to time by different workers but they have met with little acceptance.

FORMOL-GEL TEST

One of the commonly used methods for the diagnosis of camel trypanosomiasis (*T. soudanense* infection) in Africa is the formol-gel test. It is briefly described here for the convenience of field workers in India, who may not have ready access to literature on the subject The test, according to Knowles (1927*a*), is performed by adding 2 drops of commercial formalin to 1 c.c. of the suspected serum. The mixture is left for 24 hours. The reaction is regarded as positive or weakly positive in accordance with whether a complete or incomplete gel is formed. It is negative if the serum remains fluid after 24 hours.

Hornby (1923) found it valueless for the detection of bovine trypanosomiasis in Central Africa.

Spackman 1923) tested the value of formol-gel method on 6 surra camels ; in 5 complete and in the other slight opalescence and gelatinization were obtained, while no reaction took place in the control. The formalin 'must strongly small of formalin'.

Bennett, the discoverer of the sublimate test, appears to have successfully used the formol-gel test, in his earlier work, for the diagnosis of camel trypanosomiasis. In the annual report of the Sudan Veterinary Department for 1927, he recommends the use of the test in November, that is, 2 months after the period-July—September when camels usually become infected. He, however, stresses the desirability of supplementing the test by microscopical examination.

Knowles (1924), in particular, found the formol-gel test generally reliable. A form of trypanosomiasis for the diagnosis of which Knowles (1927*a*) used the test in the Sudan is locally known as 'gufar' which is considered by him to be the same as surra and identical with what is called in N.-W. Africa 'el debab' and 'm' bori'. He concludes from these tests that healthy animals to the extent of 5 to 7 per cent may give positive reaction to the test, but that such reactions are generally temporary. He records having failed to obtain evidence to show that reaction to this test may occur in diseases other than trypanosomiasis. On the other hand, Cernaianu (1928) found the test to be positive for dourine, glanders and epizootic lymphangitis, although never for healthy animals. He describes the test as a valuable accessory diagnostic method ('wertvoller diagnostisches Hilfsmittel').

Referring to dourine in Albania, Carpano (1939) states that he found the formol-gel test reliable for the diagnosis of the disease.

Gusev *et al.*(1940) carried out the formol-gel test *pari passu* with microscopical examination for surra in as many as 4311 camels in the Kara-Kalpak Republic. Trypanosomes were found in smears from 26 cases, while formol-gel test gave positive or doubtful results in 97 cases ; only in 4 cases were the results of both positive. These workers, therefore, regard the formol-gel test as not being specific.

It is of interest that Brenner (1924) applied the serum aldehyde test (same as formol-gel test) in 10 cases of sleeping sickness and all proved positive ; 7 normal persons did not react.

Morrison (1924) has tabulated 20 human cases, of which 13 showed positive reaction to formol-gel test. Of these 13, 4 suffered from other diseases and he, therefore, does not attach a 'definite diagnostic value' to the test.

Gutierrez (1928) records 80 per cent positive cases with formol-gel test in human trypanosomiasis. Cockson (1947) also appears to have obtained some positive results with this test in the same disease. He considers the test to be of 'distinct value' and especially so 'where refinements of diagnosis are impossible'.

Dye (1926) found that the formol-gel test was of very limited value when used for the detection of *T. rhodesiense* infection Ledentu and Vaucel (1927*a*) likewise used it with little and partial success in the diagnosis of the disease amongst Africans and Europeans respectively.

That the serum formalin reaction is not specific for trypanosomiasis appears to have been conclusively established by the fact that Johnson (1925) found a large number of syphilis patients in Nigeria reacting positively to the test. Thus, out of a total of 168 sera from infected cases, as many as 84 were strongly and 24 weakly positive. The sera were also subjected to the Sachs-Georgi test and the results, as compared with those obtained with the serum formalin test, were as follows : with the Sachs-Georgi test, 99 cases were strongly and 28 weakly positive, while 41 were negative. Of these, 80·8 per cent , 53·6 per cent and 31·7 per cent gave positive serum formalin reaction.

Ledentu and Vaucel (1927*a*) likewise found the test to be non-specific, being also positive in cases of syphilis. Contrary to what one would expect, they found that, in human trypanosomiasis, the reaction took seven minutes to take place in the first-stage untreated patients, 25 minutes in the second-stage patients and 2 hours in advanced cases. Ledentu and Vaucel (1927*b*) concede that both agglutination of red cells (*infra*) and formol-gel test are useful aids for the diagnosis of trypanosomiasis and they produce some figures to show that they are almost of equal rank in respect of reliability. According to them, the absence of auto-agglutination always corresponds with a negative formol-gelification. Wilson (1930) likewise regards these tests as of equal value although neither is specific.

The reaction rate of formol-gel test (also called 'Reaction of Gate') is stressed by Hope-Gill (1938; *cf.* Ledentu and Vaucel, 1927*a*) as an index for the diagnosis of *T. gambiense* infection. The reaction is strongly suggestive of positive infection if it takes place in 60 minutes ; otherwise, there is strong likelihood of infection being absent. Infection may be declared as being almost certainly absent if the reaction does not occur in 24 hours. It is retarded in cases previously treated with suramin or tryparsamide.

MERCURIC CHLORIDE TEST

The mercuric chloride test, developed by Bennett and Kenny (1928), is claimed by them to have been used with consistent success in the diagnosis of camel trypnosomiasis. The test is described by them as follows: 'one drop of serum is added to 1 c.c. of 1-20,000 or greater dilution. A positive reaction is detected by an opacity and a negative reaction by no change at the end of a quarter of an hour'. In one instance, they applied the test to 56 camels, side by side with the formol-gel test and microscopical examination of blood smears. The results were as follows: 3 of the animals proved positive to all the three tests; 14 positive to microscopical examination and mercuric chloride; 3 positive to formol-gel and mercuric chloride; 2 positive only to mercuric chloride; 34 negative to all 3 tests, while none negative to mercuric chloride proved positive to any other test. The failures were 5 times in microscopical examination, 16 times in formol-gel test and twice in both, but mercuric chloride test proved positive in all these. The mechanism of mecuric chloride reaction has been discussed by Horgan and Bennett in an article published by them in 1929. According to them, 'the reaction is referable to the relative and absolute increase of the euglobulin of the serum.'

Bennett (1933*a*), referring to *T. evansi* infection in camels in Sudan, claims that the mercuric chloride test is not only superior to the formol-gel test but is more reliable than microscopical examination.

Coronel (1933) states that he applied the test to 14 buffaloes infected with surra and 13 of these proved positive and this was confirmed in each case by microscopical examination. The remaining one was negative to both the sublimate test and smear examination. Tchernomoretz (1933) testifies to the accuracy of the method in *T. equiperdum* in guinea-pigs and mentions it as being 'clear and reliable'.

On the other hand, according to Manley (1929),the test is valueless for cattle trypanosomiasis. Nieschulz and Wawo-Roentoe (1929) likewise report having found the testineffective for cattle surra. Krijgsman (1930), although conceding that the sublimate test may be of some use for the diagnosis of surra in camels, does not consider it as specific for the disease, for one of the factors that cause flocculation of the serum proteins characteristic of the test is the concentration of the lactic acid and this occurs in a number of pathological conditions.

Hall (1930), referring to *T. congolense* and *T. vivax* infections in Nigeria, reports having found the test unsuccessful in cattle and goats and the adhesion phenomenon (*infra*) only occasionally effective in *T. congolense* infections.

Kuppuswamy (1941), referring to *T. evansi* infection in cattle and goats in Penang, states that both the sublimate and the formol-gel test are 'of doubtful value' for the diagnosis of this disease.

The results obtained by Randall (1935) from the use of sublimate test in equine surra in the Philippines showed that the test was unreliable and in any case much inferior to complement-fixation test. Thus, with the latter, prositive or 'suspicious' results were recorded in 47 infected animals and negative in 20 controls. With the mercuric chloride test, 18 positive, 16 'suspicious' and 11 negative results were recorded in infected cases and one positive in a control animal. As a matter of fact, it may be declared that repeated trials have shown that the reliability of the complement-fixation test for the diagnosis of trypanosomiasis still remains unchallenged.

Van den Branden (1935), too, found the sublimate test (called by him 'Foulton's Reaction' not sufficiently specific as a means of diagnosing *T. congolense* and *T. brucei* infections in rats. De Georgi (1936) likewise found the test (called by him 'Foulton's Mercuric Chloride Test') as being of no practical use in the diagnosis of *T. evansi*, *T. brucei* and *T. pecaudi* infections in white rats.

Steevenson (1938) in Waziristan (Pakistan) applied the sublimate test in an outbreak of camel surra and found that in some cases the test was not positive until two weeks after infection, while in animals that had recovered after suramin treatment, the test continued to be positive for about a month. He recommends that for the study of flocculence 'the tubes should be held against a dark background up to a strong light.' According to Jones (1936), nitric acid test is definitely specific for trypanosomiasis, although he mentions Bennett and Kenny as having called it non-specific.

COMPLEMENT-FIXATION TEST

The complement-fixation test is perhaps the most dependable of all the diagnostic tests so far devised for trypanosomiasis, although in veterinary work, the laborious method necessary for carrying it out makes it unsuitable for large-scale use under field conditions in India and, possibly, also in most countries where animal trypanosomiasis is prevalent to any large extent. The test has been adopted during recent years on a fairly extensive scale in the Philippines (Kelser, 1927), while in India, it has been employed by the Mukteswar Institute with uniform success.

The test appears to have been used successfully by Chu-Jen-Ku (1926) for the diagnosis of what he calls 'mal de coit' (evidently dourine). The reaction in the case of rabbits is stated by him to have been regularly obtained ('regelmassig festgestellt').

Robinson (1926) studied the comparative values of complement-fixation, Sachs-Georg and Meinicke Lipoid-fixation tests in the diagnosis of dourine and nagana. The last is essentially a flocculation test and involves the use of 'alcoholic horse heart extract and dourine antigen.' The first proved to be the most dependable. De Kock *et al.* (1939) have shown that, in dourine, even chemotherapeutic treatment does not influence reaction to the complement-fixation test. They found the test to be 'very consistent.'

ADHESION PHENOMENON

The adhesion phenomenon, as defined by Davis and Brown (1937), 'is characterized by the adhesion to the parasites, when acted upon by the immune plasma *in vitro*, of blood platelets and other bodies.' It may, therefore, be used in the discovery of reservoirs of infection. As far back as in 1917, Wehrbein developed what he called 'conglutination' method for the detection of dourine, the method being described by him as closely resembling deviation of complement test ('durch zuführen als die ubliche Komplementablankungsmethode'), except for the fact that it was the clumping of the blood corpuscles, and not haemolysis, that served as an indicator in the final reading. 'Agglutination' and 'disagglutination' of red corpuscles and also 'pseudo-agglutination' were three other diagnostic methods described by Marty (1917) for the detection of trypanosomiasis. 'Auto-agglutination', described by Hornby (1919*a*), is more or less a similar phenomenon. According to him, it is generally possible, on the basis of the phenomena of agglutination and disagglutination, to diagnose trypanosomiasis ('la recherche de l'agglutination avec son cortége deglobulides permit de diagnostiquer sans difficulté la plupart des trypanosomiases').

Rickenberg (1917) was the earliest to record the phenomenon of adhesion as understood at the present time. He noticed that, when a rat was infected with nagana from mice and then cured with tartar emetic or any other drug, and 3 to 4 days later, when a drop of blood from the rat was mixed in a 2 per cent citrate broth with trypanosomes from the mice, not only the usual agglutination occurred, but in addition, platelets adhered to trypanosomes, thereby making them immobile. No such adhesion occurred when the platelets were from a non-immune animal. The phenomenon occurs only with a homologous strain and is therefore specific. It is called by some workers 'Rickenberg Phenomenon'. The so called 'red-cell adhesion'—'thrombocytabarin' of Leupold (1929)—is to be regarded as an extension of 'Rickenberg Phenomenon'. Leupold gives an instructive plate of 2 illustrations, one in cover-glass preparation of fresh blood and the other in Giemsa-stained blood smears.

Johnson and Lester (1929) consider the adhesion phenomenon as of real value for the diagnosis of human trypanosomiasis and much superior to formol-gel test. They observed that the reaction did not occur with the sera of subjects not naturally susceptible to the strain of trypanosomes against which the sera were tested. Thus, 32 human sera gave negative reaction when they were tested against *T. brucei* or when human or rat sera were tested against *T. congolense*. With chronic cases of trypanosomiasis the test gave positive results. The sera from 200 cases of sleeping sickness were tested, with positive results, against *T. gambiense*, while three cases of syphilis and seven normal cases gave negative results. Sixteen cases of sleeping sickness gave positive results with a strain of *T. gambiense* but negative results with a strain of *T. brucei*. Regendanz and Jurukoff (1930) have published an useful article on the adhesion phenomenon. According to them, such adhesion originates through 'Tektinabsonderung' of trypanosomes following the influence of immune sera on them.

The observations recorded by Saunders (1931) cast some doubt on the dependability of adhesion pehnomenon for diagnostic purposes. He applied the test in cases of sleeping sickness in Gold Coast and noted the occurrence of partial adhesion in 91 per cent of them, while completely negative results were obtained in 75 per cent of the cases which were considered free from the disease. According to him, the positive results obtained in the 25 per cent of the possibly non-infected cases may be regarded as being due to the test being non-specific or 'to sub-infective inoculations' or to mildly relapsing infections. The compiler of the present monograph, working in collaboration with his colleague Mr. Krishna Iyer, made fairly extensive observations on this test as a means of diagnosing experimental surra, but it did not prove of value.

The mechanism of red-cell adhesion and its diagnostic value have been discussed by Davis and Brown (1927), Duke and Wallace (1930), Wallace and Wormall (1931), Brussin and Kalajev (1931), Brown (1933) and Wormall (1933). According to the last named worker the test, when positive, indicates trypanosomiasis, but no conclusions can be drawn when it is negative. According to Wallace and Wormall (1931), the red corpuscles, "under the influence of a hypothetical substance 'adhesin', adhere to the trypanosomes and in a strongly positive reaction all the trypanosomes are completely enveloped with red cells." Duke and Wallace (1930) found the phenomenon to be 'irregular and uncertain'. Wormall recommends certain particulars in the technique to be adopted in applying the adhesion test. Brown and Forbes (1933) describe a case of sleeping sickness which, although clinically positive, failed to show trypanosomes in the circulating blood. It gave a positive reaction to red-cell adhesion. In a 13-page paper, Brown and Broom (1938) have published the results of further studies on red-cell adhesion and have also supplied some useful suggestions for conducting the test. They recommend what they call 'standard concentrations' of red cells and trypanosomes. Human red cells are washed three times in normal saline and the trypanosomes are also similarly washed. They are then standardized by haemocytometer.

Several other tests have been described from time to time for the diagnosis of trypanosomiasis. Of these, mention may be made of Bordet-Georges Reaction, which is referred to by Bessemans and Leynen (1923) as dependable for the diagnosis of dourine. ('un excellent moyen de diagnostic'). Van Saceghem devised what he called the 'trypanolein' test, but Velu *et al.* (1924) and Curasson (1925) found it valueless. Velu *et al.* describe trypanolein as 'un antigene special'. Its application is stated by them to result only in a slightly painful ('douloureuse') tumefaction.

De Almeida (1928 *a*) describes what he calls 'Takata-Ara Reaction' for the diagnosis of advanced cases of sleeping sickness with cerebrospinal involvement. The test would appear to be as follows: to 1 c.c. of the cerebrospinal fluid is added (*a*) 1 drop of 10 per cent solution of sodium carbonate and (*b*) 0·3 c.c. of a freshly prepared mixture of equal parts of 0·5 per cent solution of mercuric chloride and 0·02 per cent fuchsin. These additions are stated by him to result in a flocculation reaction, which takes place in 3 minutes in untreated and in about 30 minutes in treated cases, but in the latter the reaction may also be negative. The test is also stated to give positive result in nervous forms of syphilis.

It would appear that sera of human subjects affected with trypanosomiasis have the inherent property of causing flocculation under a variety of circumstances. Thus, Sicé (1929) demonstrated that such sera precipitated alcoholic extracts of heart muscles of sheep and goat infected with nagana. The sera were obtained by 'coagulation spontanée' and keeping at 12°C. for 2 hours.

A less known diagnostic test for human trypanosomiasis is referred to by Le Gac (1930) as 'Targowla's Reaction'. It consists in the successive placing in 'un tubes á hémolyse' of 5 drops of distilled water, 15 drops of suspected cerebrospinal fluid and 5 drops of paregoric acid (tinctura camphorae composite). In positive cases precipitation is obtained by agitating the mixture.

Jones (1936) tested 20 chemicals for the serological diagnosis of cattle trypanosomiasis and found that nitric acid produced the best results, only six having been found by him to be weakly positive out of 111 cases believed to be free from trypanosomiasis. The test was apparently unsatisfactory for *T. congolense* and *T. vivax* infections, but with *T. brucei*, *T. rhodesiense* and *T. gambiense* infections 75 per cent positive results were obtained. Generally, the number of positive results obtained in trypanosomiasis was 45 to 50 per cent. The reaction chiefly occurred in chronic or latent infections and seldom in the early stages when trypanosomes were found in the blood.

Van den Branden (1937) describes that he calls Formol-Neostibosan and Sulpharsenol Reactions in the diagnosis of animal trypanosomiasis. Neither proved good for the detection of *T. brucei* infection in guinea-pigs, rats and rabbits or *T. gambiense* infection in guinea-pigs. A mention may also be made of what Guibert (1938) has called 'Pandey's Reaction', for diagnosing human trypanosomiasis; it would appear to be somewhat analogous to the Takata-Ara Reaction described by De Almeida (1928, *ante*). The suspected cerebrospinal fluid is mixed with about 7 per cent aqueous solution of carbolic acid crystals. A quantity of the mixture is poured into a tube and the colour of the meniscus in the tube—black, greenish, blue, etc.—is observed through a small window of a box in which the tube is placed.

Curasson (1940) mentions an interesting instance of what he describes as 'provoked flare-up of latent trypanosomiasis' in 5 sheep infected with *T. vivax*. In these animals the trypanosomes re-appeared in 3 hours of an intravenous injection of 0·02 gm of pilocarpine combined with a subcutaneous injection of 2-10 c.c. of milk. The author refers to the possibility of adopting this method for causing the re-appearance of dormant trypanosomes and then applying trypanocidal remedies.

A method developed by Ray (1946) for the diagnosis of cattle surra consists in the intradermal injection of a suspension of dead *T. evansi* in equal parts of glycerol and normal saline. A caliper is used to determine whether the swelling caused by the injection has reached the degree diagnostic of infection. Ray (1950) also developed an altogether new method for the detection of bovine surra. The method is briefly as follows. A drop of serum, separated 48 hours previously from the suspected blood and inactivated at 55°-56°C., is gently discharged from a pipette on the surface of 0·5 ml. of a freshly prepared 10 per cent aqueous solution of stilbamidine (‘M & B 744’) contained in an agglutination tube. The degree of positive reaction is in the following decreasing order: (1) when the drop immediately coagulates and sinks to the bottom in a large mass and dissolves in 5 to 10 minutes; (2) when it sinks in large lumps and slowly dissolves; (3) when it sinks in small shreds and also slowly dissolves. In cases of negative reaction, the coagulum forms a ring on the surface and dissolves there in about 2 minutes. The test gives negative reaction in equine surra and inconclusive results in buffalo surra.

A reference may be made here to what Brumpt (1914) calls xeno-diagnostic method of detecting trypanosomes. The method consists in culturing suspected material in natural or vicarious vectors (usually non-vertebrate). Thus, Brumpt succeeded in culturing trypanosomes in clean leeches fed on fish suspected for trypanosomiasis but negative for the presence of these parasites on direct examination. In a note appended to Brumpt's article, Mesnil records having used this test for the detection of ‘infection latent soupconnee’ in ruminants by feeding *Glossina* on them and then on susceptible animals. In India, the method is likely to prove of some value for the detection of latent forms of *T. evansi* if and when the invertebrate host of this species of trypanosome is definitely known.

An interesting form of what may be called prognostic, rather than diagnostic, method for the clinical determination of the degree to which trypanosomiasis has advanced has been described by Schern (1925) for nagana, dourine and sleeping sickness. When blood of healthy rats or horses is added to infected blood containing non-motile (‘unbeweglich’) trypanosomes, the latter regain their activity and the duration of this activity is, according to him, an index of the period for which the infected animal is likely to survive. The observation on the trypanosomes is made on a coverslip preparation of the blood.

CHAPTER II

Arsenicals

INTRODUCTORY

A PERUSAL of the history of chemotherapy of trypanosomiasis, ever since this form of treatment was devised by Ehrlich, shows that the class of drugs that has attracted the largest amount of attention from workers in this field has been arsenicals. Ehrlich himself devoted most of his researches to exploring the possibilities of these drugs in combating what may perhaps be considered, even to this day, as the most baffling of human diseases, namely, sleeping sickness. The most notable member of this group of chemicals has been atoxyl, which has enjoyed a degree of popularity perhaps not equalled by even such drugs as tartar emetic and suramin. The work carried out with atoxyl has been sufficiently extensive to warrant a separate section being devoted to its treatment. In the present section, the results reported to have been obtained with arsenicals other than atoxyl will be briefly considered. Separate sections will also be given to the more recent arsenical drugs, tryparsamide and melarsen, while a brief chapter will be devoted to a consideration of the once well-known drug arsenophenylglycin. Arsenicals have been administered, as a rule, intravenously and other routes have only been occasionally used. Poincloux (1925) claims to have obtained recovery by the use of vapour of arsenic in mice infected with nagana.

It is useful to remember that, as with other trypanocides, the therapeutic effect of any particular arsenical often differs with the species of trypanosome against which it is used. The truth of this remark is well illustrated by the fact that, while Giemsa claimed that the arsenical 'Hochst 4002' was highly effective against *T. brucei*, *T. equiperdum* and *T. rhodesienese* in mice, Lourie (1931) found it vlaueless against *T. gambiense*, its prophylactic value being 'negligible'.

Slight variation in the chemical composition of an arsenical has been found to bear a relationship to its trypanocidal action in different species of parasites. Thus, Rothermundt and Burschkies (1939) have shown that the arsenious acid compounds with the composition 4-amino-2-oxyphenyl are more effective against trypanosomes, while those with the composition 3-amino-4-oxyphenyl have a more powerful reaction on syphilis.

In dealing with arsenicals, a reference should first be made to Loeffler and Ruhs solution which, at one time, constituted a popular remedy for several forms of animal trypanosomiasis. Beck (1910) used it successfully, by the subcutaneous route, against *T. gambiense* infection in mice, although he preferred a combination of atoxyl and antimony to this solution.

Next to atoxyl, the arsenical that has received the greatest share of attention—in human trypanosomiasis, at any rate—is perhaps salvarsan or '606' which, it may be mentioned, is merely the trade name for arsphenamine (diamino-dihydroxy-arsenobenzene dihydrochloride). Yakimoff and Kohl-Yakimoff (1910) found that, when it was injected subcutaneously into rats infected with *T. gambiense*, sterilization was effected in from 45 to 65 minutes. In a private communication to these workers, Ehrlich himself reported having obtained cures in all animals when injected on the first day of infection and in 50 per cent of the animals on injection on the second day of infection, the dose used being 0·02 to 0·03 gm. per kilo. Riquier (1911), in his observations on *T. brucei* and *T. equiperdum* infection in rabbits, found the so called 'innocuous dose' of salvarsan to be 0·015 gm. per kilo.

A distinct improvement on salvarsan resulted when Ehrlich produced neosalvarsan, for, as Kersten (1912) observes, the latter is more soluble in water, while its proportion in arsenic to that of salvarsan is 2 to 3, that is, 1 gm. of salvarsan is equivalent to 1·5 gm. of neosalvarsan, so that it is less toxic. Kersten tested both drugs on nagana in mice and found that the curative concentration of neosalvarsan was 1: 10,000, against 1 : 4,000, of salvarsan; neosalvarsan is, therefore, to be regarded as a more effective drug. Against *T. equiperdum* in rats, Schamberg *et al.* (1920) found neoarsphenamine (neosalvarsan) safer than arsphenamine. It is noteworthy that, according to Gruhzit (1935), the arsenoxide mapharsen (meta-amino-para-hydroxyphenylarsine oxide) is as effective as neo-arsphenamine against *T. equiperdum* in rats.

Frosch and Knuth (1914) tested the possibility of increasing the efficacy of salvarsan for experimental nagana in the horse by combining it with optoquinine (aethylhydrocuprein) and sodium salicylate, but found these combinations ineffective. They used the last two ‘als Adjuvantien des Salvarsan’.

Lafont and Dupont (1915) testd the comparative efficacy of atoxyl, salvarsan, neosalvarsan, ludyl and galyl for *T. rhodesienese* and *T. gambiense* infections in white rats when these drugs were used in doses of 0·15 cgm. per kilo.; only the last 4 proved effective (‘nettenent supérieure’). The 4 arsenobenzenes were found to be 10 to 15 times as effective as atoxyl. The same workers (1914) had earlier isolated *T. cazalboui* from a goat and tested on this parasite, *in vitro*, the effect of all the 5 trypanocides mentioned above; atoxyl was found to be without effect, neosalvarsan exerted some reaction but proved to be inferior to the other three.

Seidelin (1915) obtained from Ehrlich a salvarsan-copper compound—so-called ‘K_3’—for test on rats and guinea-pigs infected with *T. brucei*; it was found encouraging in the treatment of this infection. He found the drug ‘powerful’ but the results were not ‘conclusive’ as to its therapeutic value.

Van den Branden (1916) gives some figures on the comparative efficacy for human trypanosomiasis of salvarsan and neosalvarsan and certain metallic combinations of the former. There were 4 trypanocides and the efficacy of each of them was determined by the duration of sterilization caused by it in 4 patients when the drug was injected at a specified dose.

Kuhn (1927) records the interesting observation that when mice infected with dourine were fed daily for five days with 1 mgm. of neo-salvarsan, a complete suppression of the disease resulted.

A derivative of diaminoarsenobenzene prepared by Oeschlin, and named by him ‘O_1’, was tested by Laveran and Roudsky (1914) by subcutaneous injection at 5 mgm. on mice infected with *T. brucei*, *T. rhodesiense* and *T. dimorphon*. The drug proved to possess high sterilizing power, but observations do not appear to have been made for the duration of sterilization. The same drug proved to be of little value when tested by Laveran (1915) on dogs and guinea-pigs infected with *T. gambiense*, *T. congolense* and *T. soudanense*. ‘Ok_1’, a derivative of arsenobenzene, was found by Mesnil and Motais (1915) to be more stable than arsenophenylglycin and also effective against *T. gambiense* in mice and rats. Both ‘O_1’ and ‘Ok_1’, in doses of 0·3 gm., were tested by Aubert (1915) on sleeping sickness with encouraging results.

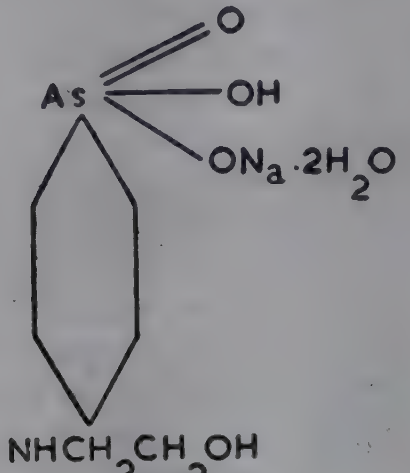
Danysz (1914*a*), who worked fairly extensively with arsenicals in the treatment of trypanosomiasis, tested on surra-infected mice the relative trypanocidal action of dioxyaminoarsenobenzol (abbreviated ‘ddab’), ‘ddab’ chloro-argentate (‘ACA’) ‘ddab’ bromo-argentate ('ABA') and 'ddab' iodo-argentate ('AIA'). When tested by a single injection on the third day of infection, 'ABA' proved distinctly superior to 'ACA' and 'AIA', a dose of 0·07 mgm. of the drug being curative for the infection. It was 3 times as active as 'ddab' and 35 times as active as atoxyl. He made the two notable findings that a number of small doses were more effective than a single large dose and that the curative dose for the infection, when the parasites were numerous in the blood, was 5 or 6 times that required if the drug was administered during the first 24 hours of infection. For surra in mice, he (1914 *b*) gives 1, 3, 8 and 35, respectively, as the therapeutic index of atoxyl, arsenophenylglycin, chlorhydrate of dioxydiaminoarsenobenzol and sulphate of bromoargentic dioxydiaminobenzolate. Danysz (1914 *c*) also tested on *T. evansi* the efficacy of '102''—a product prepared by him by combining antimony with arsenobenzol bromoargentate and having the formula $(C^{12}H^{12}O^2N^2As^2)^2$ $AgBrSbO(H^2SO_4)^2$. Cure was obtained in 3 out of a batch of 5 mice. Possibly, '102^1' is equivalent to what is known as luargol. In experimental dourine in mice, Yakimoff and Wassilesky (1917) found Danysz 102—an organic arsenical with antimony, bromine and silver—effective when used in the dose of 1·1 mgm.; its therapeutic index was 3 and, therefore, better than that of salvarsan. Yakimoff and Wassilesky used luargol in a solution of 1 in 600 to 1 in 900 in the treatment of *T. equiperdum* infection in mice. The curative dose was found to be 1 c.c. of a 1/900 solution or 1·1 mm. No relapse was observed during a post-treatment observation period of 60 days. They (1921) mention what they call 'dissodoluargol' of Danysz (on account of its better solubility) as being superior to luargol. They give 1 c.c. of a 0·5 per cent solution of this drug per 20 gm. as being lethal for the mouse and the tolerated dose as 1 c.c. of a 0·4 per cent solution, the active dose being 1 c.c. of a 0·11 per cent solution.

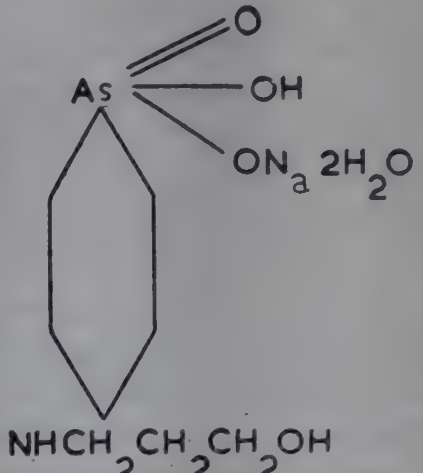
The drugs arsenic metolein (containing 8 per cent arsenic) and antimony metolein (containing 20 per cent antimony) were tested by Aubert and Michelli (1915) with highly encouraging results against *T. gambiense* and *T. dimorphon* infections in guinea-pigs; both the drugs were injected intramuscularly.

An observation of significance is recorded by Fourneau (1921) regarding the relative chemotherapeutic value of trivalent and pentavalent arsenicals. Contrary to the view expressed by Ehrlich and accepted by most workers that the former are superior, Fourneau cites the instance of oxyaminophenylarsenic acid (the so called '189') prepared by him and found highly effective in the treatment of experimental trypanosomiasis. As for the alleged nervous symptoms produced by arsenic acids, he considers it likely that such symptoms are due to impurities. This preparation was tested by Navarro-martin (1922) on *T. rhodesiense* and *T. brucei* and was found to exert a marked trypanocidal effect against these parasites when injected subcutaneously. Its therapeutic co-efficient is stated by him to be superior to that of all known arsenicals. In a paper published later in the same year (1922), Navarro-Martin, working in collaboration with Stefanopoule, records the results of some tests made with this drug on guinea-pigs infected with *T. brucei* and *T. gambiense*. The maximum tolerated dose was found to be 0·25-0·3 gm. per kilo., the curative dose being one-third the minimum lethal dose. The drug was found by them to be 'parfactement tolerées'. Blanchard and Lefrou (1922) used '189' in the treatment of sleeping sickness. A medium dose of the drug effected a temporary sterilization, while a large dose, although highly effective, caused marked local reaction.

The drug orsanine was tested for toxicity by Launoy (1939) on mice; it was found that a single dose of 4 mgm. was lethal for 11 out of a batch of 20 mice tested upon, while 3 doses of 1, 1·5 and 1·5 mgm. at 3-4 hours' interval proved lethal for 5 out of 20 mice. A single dose of 3 mgm. was found to be curative for *T. brucei* infection.

Two other pentavalent arsenicals may also be briefly referred to. These are the monosodium salts of 2-p-arsono-anilino-ethanol (etharsanol) and 3-p-arsono-anilino-propanol (proparsanol). Their constitutional formulae are given below as being illustrative of the difference in chemotherapeutic efficacy caused by the slightest difference:





Stratman-Thomas and Loevenhart (1928), who produced these two drugs, tested them on *T. brucei*, *T. equiperdum*, *T. equinum*, *T. lewisi* and *T. rhodesiense* in rats and guinea-pigs. The therapeutic dose of the drugs—0·35 gm. intravenously—was about the same as the lethal dose (*cf.* atoxyl), due to the fact that both drugs were excreted quickly. Three doses of 0·25 gm. of etharsanol on alternate days produced no bad effects. Etharsanol proved superior to proparsanol, and according to them, it 'produces no deleterious symptoms until doses approaching the lethal are reached.'

Reference may also be made here to the pentavalent arsenical *a*-4-arsenophenylglycylmonoglyceride, which has been shown by Cantrell and McGeachin (1951) to be trypanocidal for *T. equiperdum* in mice, although it is less active than tryparsamide.

Possibly, one advantage, at any rate, of trivalent over pentavalent arsenicals is that several compounds of the former category have a lesser arsenic content. Thus, only one-tenth of the arsenic in tryparsamide and other pentavalent arsenicals is contained in the trivalent arsenical amino-phenyl arsenothiomalate of soda, which was found by Launoy (1935) to produce sterile recovery in rabbits and guinea-pigs when the drug was injected at a dose of 5 cgm. per kilo.

Adler (1921*a*) tested the trypanocidal effect of phenylglycine amide-arsenate of sodium on *T. brucei* and *T. rhodesiense* in rats, and mice; the minimum lethal dose of this drug worked out at 1·2 gm. per kilo. At a dose of 0·7 gm. per kilo., it proved effective against *T. brucei* but not against *T. rhodesiense*. He mentions the high minimum lethal dose as a 'remarkable feature' of the drug. Nicolau *et al.* (1925) report having made successful use of basic acetyloxyaminophenylarsenate of bismuth in treating nagana in mice. Forty-eight hours after infection, they injected the drug, by the subcutaneous route, at a dose of 5 c.c. of an oily suspension containing 0·041 gm. bismuth and 0·15 gm. arsenic per c.c.

Levaditi *et al.* (1926) tested the relative merits, as prophylactic, of the four phenylarsenic compounds tryparsamide, 'Fourneau 270', stovarsol and 'Fourneau 192' against experimental nagana in rabbits. The first two proved to be more effective, a dose of 0·1 gm. per kilo. being protective when injected 1-2 days and 2 days, respectively, before a conjunctival inoculation with the parasites.

Between tryponarsyl (tryparsamide) and 'Fourneau 270', the latter was found by Van den Branden (1934) effective when tested at a dose of 0·05 gm. against *T. congolense* infection in white rats, while tryponarsyl was ineffective even at a dose of 0·2 gm. In view of the claim made by certain workers that urotropine (hexamethylene-tetramine) enhanced the therapeutic effect of arsenicals in sleeping sickness, Van den Branden (1934), working in collaboration with Pottier, tested this combination against *T. congolense* infection in rats, out it proved to be not better than either used alone.

A reference should also be made here to the arsenical known as neocryl or 'S.107' (sodiumsuccinanilomethylamide-p-arsonate) prepared by Morgan and Walton (1931, 1937)- Yorke *et al.* (1936) found that the maximum tolerated dose of this drug for a mouse weighing 20 gm. was 40 to 50 mgm., while the minimum curative dose for *T. rhodesiense* infection was 12·5 mgm., as compared with 20 mgm. of tryparsamide. Lester (1935) mentions the drug as having been used in 8 cases of sleeping sickness with encouraging results, including some amelioration of the cerebrospinal symptoms.

Strangways (1935) mentions the 2 arsenicals 'K.324' and 'K.352' as having been found effective against *T. equiperdum*, *T. rhodesiense* and *T. gambiense* in mice and rabbits. 'K.352' is stated to be Di (3-carboxy-3-aminoethyl) benzamide-p-thioarsenite and 'K.352' Di glutathionyl-4-acetamino-2-hydroxyphenyl thioarsenite. In *T. rhodesiense*, recovery was obtained with a single dose of 'K.352 at 0·04 gm per kilo. or when it was administered in 4 to 8 doses of 0·01gm. per kilo.; 'K.324' produced similar results. Like most other remedies, both these arsenicals proved to be entirely non-effective against *T. congolense*.

The phenyl arsenoxide oxophenarsine or mapharsen (3-amino-4-hydroxyphenyl arsenious oxide or the so called 'azo'), when used in repeated subcurative doses,was tested by Swinyard *et al.* (1942) on *T. equiperdum* in rats. The special interest attaching to their observations consists in the fact that they found that, when treatment was commenced 24 hours after infection, 2 or more doses were curative at any interval up to 96 hours. Eagle *et al.* (1944) tested 54 derivatives of phenyl arsenoxides and 9 phenyl arsonic acids on *T. equiperdum* in mice and found p-arsenophenyl butyric acid to be the most promising of these. The sodium salt of γ-(p-arsenophenyl) butryic acid is, according to Eagle (1945), curative for trypanosomiasis of horses and sheep (presumably *T. equiperdum* and *T. rhodesiense* infections), but not of cattle. The drug is also claimed by him to have been used with encouraging results in 200 cases of sleeping sickness at doses of 0·25-0·5 mgm. per kilo. 2 to 3 times weekly or even daily until a total of 6–24 injections were given. Arsenophenyl butyric acid —so called '70A'—was used by Weinman (1946) in 10 cseas of human trypanosomiasis: those in the early stages were negative up to 11 months but the advanced cases proved refractory to the drug, which was used in 40 mgm. doses, thrice weekly, until a total of 300-400 mgm. had been given.

A number of arsenical preparations of undisclosed chemical composition, and bearing various symbols, have been introduced into the field of chemotherapy of trypanosomiasis. Thus, Giemsa and Mayeda (1927) mention 'B.R.68' as having been found, when tested on nagana in mice, to have a therapeutic index of 1 : 25, as compared with 1 : 2 in tryparsamide and 3 : 1 in atoxyl. Presumably, this is an arsenical pyridin preparation similar to 'B.R.34' and 'B.R.34*a*' which have been found by Collier and Krause (1929) to be trypanocidal for *T. brucei* in mice. Collier (1930) also mentions 'B.R.34*a*' as highly effective against *T. gambiense* in white mice, its chemotherapeutic index being 1 : 50. Giemsa (1929. 1930) mentions a benzol arsenic acid, designated '4002' and possessing a high trypanocidal action, with a chemotherapeutic index of 1: 10 to 1 : 16. Encouraging results are also claimed to have been obtained in 5 cases of sleeping sickness when the drug was injected intravenously in repeated doses of 1 to 1·5 gm. He explains his reasons for exploring the possibilities of aromatic arsinic acids as trypanocides. These reasons are that arsinic acids, being more diffusible than arsenobenzols and other chemotherapeutic compounds, held out a greater promise of proving more effective by penetrating into the cerebrospinal canal. According to Giemsa, '4002' possesses much greater trypanocidal power than such pentavalent arsenicals as tryparsamide and atoxyl. Lourie (1931), however, found '4002' valueless when tested on *T. gambiense* and *T. evansi* in rats, as mentioned earlier.

Combinations of arsenicals with non-chemical agencies have been tested by certain workers against experimental trypanosomiasis in laboratory animals. Thus Richet (*fils*) and Dublineau (1932) tested on nagana-infected mice a combination of subcutaneous injection of arsenicals with their exposure to a temperature of 37°-38°C. and 42°-45°C. —so called 'pyretotherapy"—and found that the combined treatment increased the period of survival of the animals and also reduced their mortality. Pyretotherapy alone is stated by them to be insufficient for obtaining recovery. Marzinowsky (1932) studied the combined effect on *T. equiperdum* in mice of an arsenical designated 'novarsolan' in combination with ultraviolet irradiation. All the animals received a subcurative dose of the drug and, immediately after, 14 of these were irradiated for 15 minutes, while 14 were left as controls. The latter died between the 6th and 11th day, while 12 of the irradiated mice survived 13 to 20 days (average 2·5 times the period of survival of the controls). The irradiations were done with, 'Hanauer Hohensonne' at a distance of 40 cm.

Scholtz's (1935) results point to the possibility that the reported failure to use certain arsenicals successfully against trypanosomiasis is—at least partially—attributable to these drugs having been administered in a single dose instead of fractional doses spread over a length of time. This is illustrated by the results obtained by him with salvarsan against *T. brucei* infection in mice and rabbits when the drug was used in a single injection and the same dosage 3 times at 3-hourly intervals. The latter method proved superior. Another noteworthy feature was the superiority of a diluted over a concentrated form of the drug.

Rosenthal's (1942) observations on the effect of mapharsen on *T. equiperdum* in mice are illustrative of the care with which a chemotherapeutic drug is to be combined with one or more others in the treatment of trypanosomiasis, for the use of such combinations may result in disappointment on account of incompatibility of the drugs. Rosenthal showed that, while the maximum tolerated oral dose of mapharsen for mice was 0·15 gm. per kilo., it could be raised to more than 1·0 gm. per kilo. when it was mixed with glutathione (a tripeptide of glutanic acid, glycine and cysteine) at 6·5 gm. per kilo. Again, the oral curative dose of mapharsen was 0·04 gm. per kilo.; the curative dose of the drug had to be raised to 0·06-0·08 gm. per kilo. when 6·5 gm. of glutathione was mixed with it.

In the early days of chemotherapy of animal trypanosomiasis, arsenicals in one form or other were almost the only drugs used and successes were by no means infrequent. Thus, Beal (1912) mentions having obtained recovery in 8 out of a batch of 20 equines treated with combinations of arsenic and antimony. It would appear that it was the occasional practice in those days in Africa of smearing horses with powdered tobacco in combination with 'moshi' butter and then dosing them with arsenic before they were sent to fly areas. Orpiment was apparently the commonest drug in use.

Thiroux and Teppaz (1909 *a*) successfully used orpiment alone in the treatment of '*baleri*' of horses. The drug was given as follows: 1st day, 20 gm.; 4th, 25; 7th, 30; 10th, 13th, 16th and 19th, 30; 8 days' rest; 2nd course of 5 doses at 3 days' intervals. The treatment was, applied in the very early stages of the disease. Laveran (1910) found this drug curative for *T. congolense* infection in laboratory animals. Orpiment used as an electuary, combined with tartar emetic, is claimed by several workers as effective against trypanosomiasis. Thus, De Greef (1912) records success with such combination in the treatment of *T. cazalboui* infection in a horse. He even expressed the view that the question of treatment of equine trypanosomiasis was practically solved ('pratiquement résolvé) by the use of orpiment. Mouchet and Dubois (1913) likewise found that, while orpiment alone proved dangerous in the treatment of *T. cazalboui* infection, it improved the action of emetic against this injection. In the form of a bolus or electuary, orpiment was often administered in doses of 8 to 12 gm. (Hutchins, 1915). Pecaud (1912) appears to have used with some success orpiment, alone or in combination with atoxyl or tartar emetic, in the treatment of horses and cattle infected with *T. cazalboui* and *T. dimorphon*. He found that horses were more tolerant to orpiment than cattle. He recommends the use of this drug as a bolus on alternate days commencing with 4 gm. and raising it to 7 to 8 gm. per 100 kilo. According to him, orpiment is more effective in *T. cazalboui* and tartar emetic in *T. dimorphon* infection, neither of them being of value in *T. pecaudi* infection.

Orpiment was found by Aldigé (1920) to produce a strikingly good effect in pigs infected with trypanosomes of the *brucei-pecaudi* group. The drug was used in doses of 0·5 to 1 gm. daily or at various intervals. The entire herd was later given 3 consecutive doses of 0·25 to 2 gm. and this resulted in a cessation of mortality in the herd.

Holmes (1910*a*), in his early experiments on he treatment of surra in India, held that arsenious acid was 'undoubtedly' specific for the disease in equines and he claimed to have obtained cure in 8 out of 10 animals treated with the durg in full subtoxic doses at 1-day intervals, in the form of bolus or drench. Five of these horses received 10 doses, one 8, one 5 and one a single dose. He recommends a gradual increase in the dosage in a course of treatment. He found that a single dose of the drug sterilized the circulating blood for 10 to 30 days. As for the toxic doses of the drug, he found them to be 2 to 3 gm. for ponies and 4 to 5 gm. for horses, 1 gm. of the acid in solution having proved fatal for ponies weighing 250 lb. Holmes preferred arsenious acid even to a combination of this drug with atoxyl, this preference having been possibly due to the fact that, as stated by Thiroux and Teppaz (1910*a*), horses not only bear atoxyl badly but that races of horses differ in their tolerance for this drug. On the other hand, camels, according to them (1910*b*), are better adapted to treatment with atoxyl. In reviewing his work on the successful treatment of 32 cases of equine surra with arsenicals, Holmes (1910*b*) states that horses treated with arsenious acid alone have not relapsed during a period of 7 to 14½ months, while those treated with atoxyl and arsenious acid remained free from surra during 11½ to 18 months. According to Holmes, atoxyl has no curative value but he recommends its use in order to effect an initial clearance of the circulation. Ten balls of increasing doses of arsenious acid are then given on alternate days, with administration of atoxyl whenever the parasites re-appear in the circulating blood. Holmes gives tables of the doses of arsenious acid, commencing, in the case of a large horse, with 1 gm. and increasing it to 5 gm. The treatment, according to Holmes, is likely to effect recovery in about 75 per cent of the animals 'even when the disease is contracted spontaneously and is in the last stage'.

Referring to '*souma*' (*T. cazalboui* infection) in goats and cattle in French Sudan, Dupont (1912) recommends the employment of arsenate of soda—only exceptionally but not in routine practice— in the treatment of this disease, in repeated doses of 12·5 mgm. per kilo. Sturgess (1915) in Ceylon claims having cured a case of bovine trypanosomiasis by sodium arsenite alone.

In the early days, salvarsan ranked second to atoxyl in the treatment of trypanosomiasis and this drug, or some modification of it, is still in use in medical practice. As far back as in 1912, Breisinger reported having brought about almost instantaneous disappearance ('fast augenblickliche verscheiden') of trypanosomes from the circulating blood of cattle by the administration of salvarsan, without the occurrence of toxic symptoms. Holmes (1913*a*), too, used the drug successfully in 3 cases of surra in horses. He found that a single injection of salvarsan was as good as 3 injections. According to him, the toxic intravenous dose of the drug is 0·01 gm. per lb. In dogs, the toxic dose was found by him (1913*b*) to be 0·025 gm. per lb., a dose of 0·02 gm. being well tolerated. An intravenous injection of this dose caused sterilization for 20 to 97 days. He, however, found that with salvarsan 'the period of absence of parasites from the circulation is neither regular nor definite'.

Mason (1912) reports having tested the efficacy of salvarsan on horses affected with a form of trypanosomiasis allied to *el 'debat* of camels. In a chronic case, he employed a 3-gm. dose of the drug with some improvement, but it was followed by an occurrence of relapse. Cure was obtained after a second injection of 4·2 gm. of salvarsan 2 months later. A second horse was cured following an injection of 3 gm. of salvarsan and 12 days later an injection of 100 c.c. of a 1 per cent solution of trypanblue. Mason also records recovery in a horse after it had received a course of combined treatment with arsacetin, sodium arsenate and orpiment.

It is worthy of note that salvarsan proved effective in cases where atoxyl failed, as observed by Yakimoff and Wassilewsky (1916) in the course of their work on the treatment of camel trypanosomiasis in Russian Turkestan. They described the drug as 'le meilleur reméde'.

Views held by different workers in regard to the therapeutic value of neosalvarsan would appear to have been contradictory. Thus, Dahmen (1922) found that neosalvarsan (neoars phenamine) and salvarsan were better than suramin on account of their lower toxicity. In a later paper, however, Dahmen (1926) mentions both these drugs as being unsatisfactory and expresses his preference for neo-silver-salvarsan, although even this drug was not in all cases successful ('ausreichend'). Sidalko (1931) likewise found neosalvarsan unsatisfactory for dourine. Goidsenhoven and Schoenaers (1939), referring to a Belgian product of neosalvarsan (arsenobenzyl), state that it is valueless in the treatment of the disease. They, however, consider their failure as having been possibly due to the unfavourable ('severés') conditions of their experiments, such as the abnormal increase ('pullulation anormale') of the trypanosomes in the blood. On the other hand, Prugelhef (1947) claims to have used neosalvarsan with success in 30 cases of dourine when the drug was given intravenously. He found mapharsen also effective. Kalush, as recently as in 1951, reported good results from neosalvarsan, as also from mapharsen, in the treatment of dourine.

Another arsenical which has enjoyed some popularity as a trypanocide of value is novarsenobillon. Ware (1928), in dealing with an epidemic of surra in a pack of imported fox hounds in India, used this drug intravenously, without effect, in a dose of 0·45 gm. followed, 10 days later, by a dose of 0·3 gm. Eyraud *et al.* (1936) report favourably upon novarsenobenzol as a curative for dourine. Courtade (1937) recommends the use of this drug intravensously in 2 doses of 4-5 gm. per 100 kilo., with an interval of 4 hours between them (so called Ciusa's method). Balozet (1945) succeeded in curing cases of dourine by injecting 14 gm. of novarsenobenzol, on 2 successive days, shortly after the onset of symptoms of the disease. Colin (1938) cured clinical dourine in 2 stallions by intravenous injections of novarsenobillon, on 2 successive days, in doses of 15 gm. in a 0·1 per cent solution, each dose being administered in 3 parts at ½-hr. intervals. Cordier and Menager (1938) likewise cured 2 donkeys by injecting each with 2 doses of the drug and they even concluded that it was superior to suramin.

A number of other arsenicals, which cannot very well be classified into discrete categories, have been introduced into the field of chemotherapy from time to time. A few of these may be mentioned. A form of arsenical treatment for equine trypanosomiasis, which would appear to have gained currency for some time in Nigeria, was the socalled Fagan treatment. The method recommended by Fagan is as follows (*Sleeping Sickness Bull.*, **2**, 68; 1910). Every 6 hours commencing from 6 a.m., 3 grains of powdered white arsenic in *dussa* ball made up of bran from guinea corn and native butter. Every 6 hours commencing from 9 a.m., 1 oz. drink of 1/1000 solution of perchloride of mercury in 2 quarts of water. The treatment is continued daily for 2 months and on the expiry of this period it is advisable to continue it on alternate days for another 4 months. As remarked by the Editor of the above Bulletin, it is probable that, in view of the good results reported by Holmes, Thiroux and Teppaz, the omission of mercury would have made no difference in the efficacy of the Fagan treatment. According to Andrew Foy (1911), this treatment is of value in the early stages of trypanosomiasis.

Following the discovery of the therapeutic value of arsenicals, notably atoxyl, orpiment and arsenious acid, a number of combinations of arsenic and other metals have been tested from time to time in regard to their efficacy against trypanosomiasis, but the results have not been encouraging. Danysz (1913) mentions having successfully used an arsenosilver compound against *T. evansi*, but not against *T. rhodesiense*. As mentioned earlier, Laveran (1915) tested 'O'_1'— a derivative of diaminoarsenobenzene— against *T. congolense* and 2 other species; the results were unsatisfactory.

Galyl, another arsenical, was successfully used by Curasson (1918*a*) in the treatment of 6 cases of equine '*souma*' the drug having been used by him in doses of 1 gm., combined with sodium phosphate 0·37 gm., caffeine 0·25 gm. and sodium benzoate 0·28 gm., in 5 c.c. of distilled water. Two intravenous injections were given. He extols galyl as 'd'une efficacité indéniable et d'une inocuité qu' on peut dire absoluê'.

A course of 3 injections, in doses of 5-7 gm., of the arsenical osarsan—probably a derivative of diamoniarsenobenzene—is reported by Velu (1919) to be effective for donkeys infected with *T. marocanum*. Cure was also obtained by him in 4 dogs, each injected with about 10 cgm. of the drug per kilo. at 7-9 day intervals. In the report of the Veterinary Department of Morocco for 1920, osarsan is reported to have been used with success in the treatment of *T. marocanum* infection in horses.

Van den Branden *et al.* (1927) unsuccessfully used 'Hoechst 2754' (a pentavalent compound containing 27·2 per cent arsenic) against *T. congolense* infection in 2 mules. The drug was injected subcutaneously in 8-gm. doses.

Hall (1927) tested halarsin (4oxy-3 amino-phenyl dichlorarsine), in combination with bisantol (containing 47·5 per cent bismuth and 13·6 per cent antimony), against *T.*

congolense and *T. vivax* infections in goats, but the treatment proved ineffective. He states that after repeated injections the effect of the drug was much lessened ('trés amoindre').

As recently as in 1945, Eagle claimed to have found Y—(p-earsenophenyl)-butyric-acid a most effective trypanocide amongst phenylarsenoxides (referred to earlier). When tested on mice and rabbits infected with *T. equiperdum*, its therapeutic index proved to be higher than that of tryparsamide. Eagle reports having used it with success against trypanosomiasis in sheep and a horse, but it did not prove effective against bovine trypanosomiasis.

Whatever may have been the results obtained by different workers from the use of arsenicals, these drugs would appear to be far from being such as might be depended upon, even to a moderate extent, in the treatment of trypanosomiasis. Schat (*Sleeping Sickness Bull.*, 2, 103; 1910), referring to surra, even holds that arsenicals may prolong life but cannot cure.

CHAPTER III

Arsenicals—contd.

ARSENOPHENYLGLYCIN

IN the early days of chemotherapy of trypanosomiasis, arsenophenylglycin enjoyed a degree of popularity which can be regarded as having been equalled in later years by such drugs as suramin, tartar emetic and tryparsamide. One chief reason for the readiness with which this drug was accepted as almost a specific for the disease was the fact that it was introduced into the field of chemotherapy by no less an authority than Ehrlich himself, who, as a result of numerous trials in the laboratory, came to the conclusion that it possessed uncommon trypanocidal properties. It may be mentioned here that, according to Mesnil and Motais (1915), the drug 'Ok_1'—a derivative of diaminoarsenobenzene—prepared by Oeschlin and to which a reference has been made earlier proved as effective as arsenophenylglycin when tested on rats infected with *T. gambiense.* According to Duke (1912), the drug affects trypanosomes (*T. gambiense*) in the guts of flies fed on animals treated with arsenophenylglycin. The drug was for the first time used in human trypanosomiasis by Alt (*Sleeping Sickness Bull.*, **1**, 328; 1908). Mesnil and Kerandel (1909) found that monkeys (*Macacus rhesus*) dosed at 5 cgm. of the drug per kilo.—which is one-fifth the minimum lethal dose—resisted infection with *T. gambiense.*

As explained by Roehl (1909), the discovery of arsenophenylglycin was the result of a search made by Ehrlich for an arsanilate derivative which, while being highly trypanocidal by reason of its possession of a trivalent arsenic radical, could be made non-toxic and non-irritant by the introduction of certain special groups. Arsenophenylglycin is a yellow powder, readily soluble in water and for most species of trypanosomes 2 to 4 times less toxic than arsanilates. The drug, however, has the rather serious drawback of being sensitive to oxygen, so that it has to be kept *in vacuo*, its oxidation products being highly poisonous. According to Schilling and Jaffé (1909), the drug has the further drawback of being slowly excreted, thereby remaining in accumulation for some length of time in the system. They, therefore, view with disfavour long courses of treatment with arsenophenylglycin. Tendron (1908, 1909) has also shown that, while almost all the arsenic of atoxyl is excreted from the body during the first 8 hours of its administration, excretion of arsenophenylglycin begins much later and continues for much longer time than atoxyl.

From his experience in the use of arsenophenylglycin in the treatment of a number of cases of human trypanosopmiasis, Raven (1910 *a*) concluded that the drug should not be given in advanced cases requiring prolonged treatment, that it should be given in single or double injections of not less than 0·8 to 1·0 gm. on 2 consecutive days and that, in the event of a relapse within 6 weeks of the last dose, other remedies should be resorted to. Raven (1910*b*) claims to have obtained what was permanent recovery in 18 out of a batch of 25 patients by a single injection of the drug. He recommends 1 to 6 doses of the drug for a patient. In another paper published by him in 1910 (*c*), however, he states having used arsenophenylglycin with 'tolerable' success in the treatment of sleeping sickness. Raven's results would appear to have been the best as yet recorded from the use of arsenophenylglycin in the treatment of sleeping sickness. In papers published by him in 1910 (*d*) and 1911(*a*), Raven gives 50 to 55 mgm. of the drug per kilo. as a correct dosage for human trypanosomiasis and expresses the view that failure to obtain recovery with the drug is to be attributed in many cases to insufficiency of dosage. In another paper published by him (1911*b*), Raven states having found the drug to be ineffective in cases of relapse after treatment with other remedies.

One of the reasons why arsenophenylglycin, notwithstanding its strikingly trypanocidal properties, eventually went out of use would appear to be that its effectiveness diminishes as the weight of the animal increases ; this, as stated earlier, had been observed by Breinl and Nierenstein (1909*a*). Thus, while guinea-pigs, monkeys and relatively small dogs proved readily amenable to treatment with this drug, donkeys infected with *T. brucei* were adversely affected.

The first serious note of disappointment in regard to the efficacy of arsenophenylglycin was sounded by Eckard (1910), who tested this drug on two cases of human trypanosomiasis, in doses of 0·6 to 0·9 gm. until a total 4·1 and 3·0 gm. respectively was reached in 11 days, but both relapsed. Eckard used the drug in 44 untreated cases, mostly in the first stage, and found that the resulting sterilization lasted only for a few days.

Scherschmidt (1911) likewise was disappointed with arsenophenylglycin when it was tested on human patients in 2 successive doses of 1·5 gm. for adults and 0·75 gm. for children. He considered that most of the deaths were attributable to the doses having been too large but a lower dosage had no sterilizing effect. 'The position is that either the doses must be lowered with results less good than those of atoxyl, or heightened with a still greater mortality.'

Ullrich (1911) used arsenophenylglycin in 17 cases of sleeping sickness, 2 of which were in the first stage of the disease and the others were either in this stage or had been long under treatment. The drug in these cases was given in doses of 0·3 to 0·8 gm., the number of dosages ranging from 4 to 16. Ullrich concluded that arsenophenylglycin was not superior to atoxyl and often produced some poisoning. Ehrlich's remarks, as summarized in *Sleeping Sickness Bull.* (2, 94; 1910), in explanation of the possible reason of Eckard's failure—and his remarks should apply to the failures recorded by other workers—in using the drug successfully are quoted here *in extenso* as being of general interest in regard to the rationale of the treatment of trypanosomiasis. Ehrlich 'expresses the belief that it (arsenophenylglycin) has not been employed in the best way. It is necessary, he thinks, to find out how far one can go with double injections. Two injections of 1 gm. each are the smallest quantity which should be given and he thinks it may be necessary to give more. If larger doses are found to be too dangerous, it would be necessary to associate with arsenophenylglycin another trypanocide, but he rightly insists on the necessity of first ascertaining what it will do by itself. It is clearly a mistake to estimate the danger limit of the dose of these organic arsenicals by symptoms which they may cause after many injections. The experiments of Tendron (*ante*) show that the excretion of arsenophenylglycin is slow, so that by repeated administration, although the drug is never in sufficient concentration to kill all the trypanosomes, supersensitiveness of the tissue, quickly developing, may cause symptoms of poisoning, though the individual doses have been relatively small. Or, apart from accumulation, the proportional distribution between parasites and host becomes disturbed, so that the drug is fixed by the host's tissues and less by the trypanosomes. Schilling's cases (*infra*) seem to illustrate the rational method. He gave two injections and then held his hand.' The cases of human trypanosomiasis referred to by Ehrlich above as having been successfully treated by Schilling were those of a German missionary, his wife and son 10 years old. The husband and the wife each received 1 gm. and, 3 days later, 0·5 gm. of the drug, while the boy received 2 doses of 0·5 gm. Recovery was obtained in all the 3 cases (*Sleeping Sickness Bull.*, **2**, 106; 1910).

Mesnil and Kerandel (1910) classify into 2 categories all recoveries obtained with arsenophenylglycin. In one, permanent sterilization is effected as a direct consequence of the administration of the drug; in the other, the parasites, in spite of the treatment, continue to remain in the circulating blood for some length of time until the treated animal is able by itself to acquire a relapse and in this way also acquire immunity. Cases of the second category, according to them, are of infrequent occurrence. These conclusions are stated by them to be based on the results of the treatment of experimental *T. gambiense* infection in monkeys and of 3 cases of bovine surra.

Martin and Ringenbach (1910*a*) treated 33 second or third-stage cases of human trypanosomiasis with arsenophenylglycin. They used the drug in a 5 per cent concentration, in doses of 0·5 to 4 gm. Eleven died and of the others 3 were 'decidedly improved'. They concluded that the drug, while being highly trypanocidal, had little actual curative effect; the trypanosomes, although absent in the circulating blood, were found in the crebrospinal fluid. According to them, the drug 'may be regarded as a prophylactic.'

According to Aubert and Heckenroth (1911 *a*), a suitable dose of arsenophenylglycin for use in the treatment of sleeping sickness is 5 to 7 gm. divided into two parts to be injected at 10 days' interval. They observe that, as the drug is only of value for patients who are in the first or early second stage of the disease, it has no advantage over other drugs hitherto employed Aubert and Heckenroth (1911 *b*), however, hold that prophylactically arsenophenylglycin is decidedly superior to atoxyl. In a paper published in 1913(*b*), they give particulars of **37** cases of human trypanosomiasis treated with arsenophenylglycin. Twenty of these improved, 12 were unaffected and in 5 the disease was augmented. Five of them were in perfect health after treatment. They used the drug intravenously in salt solution or distilled water, in 1 or 2 injections at intervals of 4 or 5 days, at 45 mgm. per kilo.

In a report prepared by Hodges, Van Someren and McConnel (statistician), summarizing the results obtained with arsenophenylglycin in the treatment of human trypanosomiasis in the Uganda Protectorate (*Sleeping Sickness Bull.*, **3**, 253; 1911), the average dose of the drug is stated to have been 1·5 to 2 gm., the initial dose having ranged from 0·5 to 4 gm. Hodges, while unable, in view of the shortness of the post-treatment observation period, to express an opinion on the 'positive value' of the drug, considers the preliminary indications as pointing to the conclusion that it does not possess superiority over several other drugs which are much less toxic. On the other hand, Van Someren mentions a dose of 3 gm. as having been well tolerated, without the production of toxic symptoms. He also claims to have obtained 'encouraging results' with arsenophenylglycin in cattle trypanosomiasis. He recommends the use of a heavy initial dose instead of repeated small doses.

For the detection of relapses in human trypanosomiasis after treatment with arsenophenylglycin, Hellen (1913) lays down that the patient after apparent recovery should have his temperature recorded for at least 7 months and, in the event of an elevation of the temperature without obvious cause, he should be kept under observation for another 5 months A condition, so rigorously laid down, seems hardly capable of being fulfilled, particularly when dealing with a large number of patients, so that it may be assumed that the percentage of recovery claimed to have been obtained from the use of this drug was in some instances more than what was actually the case.

Monfort (1913) records having obtained very encouraging results when the drug was injected in 3-gm. doses into mice experimentally infected with 3 species of trypanosomes as is being detailed. Fourteen animals infected with *T. gambiense* and *T. rhodesiense* remained relapse-free, after receiving the drug, during an observation period of 86 and 64 days respectively. Only one relapsed in a treated group of 5 animals that had been infected with *T. dimorphon.* The drug, however, proved ineffective against *T. congolense* when tested on seven mice infected with this species of trypanosome.

Arsenophenylglycin has only been occasionally used on animals. It was found by Schilling (1909) to exert a feeble action on nagana parasite *in vitro* but was curative for nagana in rats. A nagana horse given the drug at 0·1 gm. developed toxic symptoms but recovered, while death occurred in a seond infected horse similarly treated. Arsenophenylglycin has the exceptional property of being lethal for *T. lewisi* (Wendelstadt, 1908), which is unaffected by atoxyl and this led to hope being entertained that it would prove effective for all species of trypanosomes.

The drug was used by Strong and Teague (1910) on monkeys and horses experimentally infected with *T. evansi* and their observations are, therefore, of particular interest for workers in India. The minimum lethal dose of the drug for monkeys was found to be 0·22 to 0·26 gm. per kilo. when the drug was used in a 5 per cent solution ; the currtive dose was 0·04 to 0·09 gm. per kilo. as relapses occurred when a dose of 0·06 gm. or less was used. Strong and Teague used a dose of 0·08 gm. in a group of 20 monkeys and all except one were cured. They observed that none of the monkeys that had a dose of 0·1 gm. per kilo. or more showed trypanosomes in their circulating blood after 24 hours and in no instance did the parasites return. The minimum lethal dose of arsenophenylglycin for horses worked out at 0·052 gm. per kilo. Twenty horses in an epidemic were treated with the drug ; 9 months later seven were found to have recovered from the disease. According to Strong and Teague, the drug proved most effective when the curative dose was close to the lethal and small doses were unsatisfactory. Arsenophenylgylycin had also been previously found by others to be much less satisfactory in horses than in monkeys. According to Schilling (1909), the maximum tolerated dose for horses is 0·075 gm. per kilo.

The efficacy of arsenophenylglycin was tested for dourine by Watson (1911) and for nagana in cattle by Breisinger (1912), but in neither did it prove satisfactory. In the first, it proved to possess better sterilizing power than atoxyl, but there was a recurrence of the fever. In the second, the drug, on account of the high dose necessary, was found risky to use. Breisinger used this drug in combination with tartar emetic in the treatment of cattle experimentally infected with nagana, but its employment in practice was found dangerous ('grosser Gefahr') owing to the large dose required.

The position in regard to arsenophenylglycin is summed up as follows in an editorial comment in *Sleeping Sickness Bull.* (**3**, 259; 1911) : 'The poor results are best explained by the small number of early cases available. Arsenophenylglycin seems no better than any other trypanocide and according to some it is inferior to atoxyl in cases which are advanced or have relapsed under treatment. The lesson seems to be that the drug must be used early and in combination with one or more drugs.'

Thus, in spite of the favourable results occasionaly obtained from the use of arsenophenylglycin in the treatment of human trypanosomiasis, the drug has failed to fulfil the hopes that were entertained about its efficacy immediately following its discovery by Ehrlich, although these failures have been explained away by certain authorities on the score of faulty method of administration of the drug or of using it on wrong patients.

As already mentioned earlier, arsenophenylglycin is lethal against *T. lewisi* Kuhs *et al.* (1936) made the important observation that in this infection recovery did not depend on the number of parasites in the circulating blood but on the duration of the infection. In the view of the compiler of the present monograph, this indirectly points to the conclusion that the curative and the prophylactic dose of a trypanocidal drug should be approximately the same.

CHAPTER IV

Arsenicals—contd.

Atoxyl

ATOXYL, whether alone or in combination with other drugs, was almost invariably used in early trials on the chemotherapy of surra in India. The courses of treatment were usually prolonged, while the result could at the best be claimed to have sometimes been moderately satisfactory. The object of the present section is more to provide some indication of the methods in which atoxyl has been used by different workers in the treatment of animal trypanosomiasis, than to give particulars of the extent of success obtained by the use of these methods, so that workers desirous of carrying out tests with atoxyl may be enabled to draw up their own schedules of using atoxyl, with due regard to such reasons as might appear to them to have been responsible for failures on the part of previous workers to use the drug with advantage.

Ciuca (1914*a*) as already mentioned, developed an altogether novel method for increasing the therapeutic effect of atoxyl. He found that the duration of sterilization after atoxyl in guinea-pigs infected with nagana could be increased by previously inducing abscess formation in these animals by injecting essence of terebene and that repeated application of this combined method kept the circulation clear of trypanosomes for a long time, but a complete cure was not obtained. About a dozen injections of atoxyl, in doses of 0·015-0·02 gm., can be given to an animal without deleterious effects, the dose of terebene for each injection being 1 c.c. According to Ciuca, the result of such injection is 'un obstacle momentane à la multiplication' of the trypanosomes. He also considers it probable that 'abscess of fixation' facilitates the production of trypanotoxyl-'veritable substance active' of atoxyl. A futrher point of interest in connection with the use of atoxyl is Berte's (1939) finding that the subcutaneous injection of this drug is painless ('indolore') when it is given dissolved in 10 per cent sodium citrate solution.

Following upon reported successful treatment by Thiroux and Teppaz of experimental surra is guinea-pigs by alternate doses of atoxyl and orpiment, Holmes (1908) tested their method in India. The method was unsuccessful in 3 ponies but proved effective in 2 other ponies, 3 rabbits and 5 guinea-pigs. The doses used by him were as follows: (1) *Ponies.* Four gm. atoxyl every 4th day and 0·5 gm. orpiment in the interval, with a total of 4 doses of each. (2) *Guinea-pigs.* 0·015 gm. atoxyl and 0·009-0·0135 gm. orpiment at the same intervals as in horses. (3) *Rabbits.* 0·04 gm. atoxyl and 0·01 gm. orpiment. Holmes (1909) carried out further experiments on these lines on 7 guinea-pigs and 2 rabbits, 5 doses of each of atoxyl and orpiment being given alternately every 2nd day; 2 of the guinea-pigs and the rabbits received the treatment 3 weeks after surra inoculation, while the other 5 guinea-pigs received the treatment within 3 days after the appearance of trypanosomes. Cure was obtained in all the animals. Holmes also used the atoxyl-orpiment treatment in 11 surra ponies, 6 of which, however, died while under treatment. In one unsuccessful case, an apparent cure was obtained when the 2 drugs were used in conjunction with tartar emetic, while 2 recovered after receiving a combined treatment with atoxyl, orpiment and sodium arsenite. According to Holmes, the last combination was superior to any other form of treatment. He concluded that atoxyl and orpiment 'can effect a cure even in advanced cases' of equine surra. A similar view in regard to arsenious acid was expressed by him later (1910 *b;* see p. 31). Holmes

(1910*c*) found atoxyl valueless when given orally to horses infected with surra. He then used atoxyl subcutaneously as follows : (1) Alternately with arsenious acid in bolus, 5 doses of each being given; (2) in combination with Loeffler and Ruhs solution as drench and arsenious acid in bolus on consecutive days, repeated once or twice after intervals of 4 days. Sixteen horses were treated by each of these two methods, the recoveries being 9 by the first and 12 by the second method. From these experimental observations, Holmes concludes that arsenious acid alone, when given in full sub-toxic doses and not in small daily dosage, is a 'specific' for equine surra and is superior to a combination of atoxyl and arsenious acid. It is noteworthy that, in Holmes's view, orpiment may, for all practical purposes, be regarded as equivalent to arsenious acid, so that the recoveries obtained previously by him by using a combination of atoxyl and orpiment is attributable to arsenious acid. In a paper published by him in 1911, Holmes states that a combination of arsenious acid and atoxyl is ineffective or fatal, although arsenious acid, when administered in combination with tartar emetic, causes a temporary sterilization. The latter view had also been expressed by him in an earlier paper (1910*c*) where he further remarked: 'Arsenic appears to be an unsuitable drug for the treatment of surra in camels. Its action is uncertain.'

According to Leese (1910*a*), a combination of atoxyl, tartar emetic and sodium arsenate is the best form of treatment for camel surra, the firt two being given intravenously and the third by the mouth. He recommends the administration of these drugs on successive days, with 2, 4 or 8 days' interval between the courses. In one of his series of experiments, Leese (1912*a*) successfully used a combination of atoxyl and tartar emetic intravenously and sodium arsenate by the mouth in doses of 4-4·5 gm., 0·5 gm. and 5 grains respectively. Five doses of each were given and the treatment was spread over a period of 37 days. Three out of a group of 4 camels were cured. The cost of this treatment for each animal was Rs. 9, so that a sum of Rs. 36 was spent for the 4 animals and, therefore, Rs. 12 for each successful case, against Rs. 110 to Rs. 200 representing the cost of one animal.

Leese (1912*b*) used atoxyl in 7 different combinations, each being designated by a number : (1) *'264' combination.* Atoxyl, 4 to 4·4 gm. intravenously ; tartar emetic 0·5 gm. plus sodium arsenate 55 to 60 grains, in drench on 3 consecutive days. The combination of the 3 drugs was repeated at intervals of 2, 4, 8 and 12 days. No relapse occurred in 3 treated animals during an observation period of 253-498 days, but one relapsed in 19 days. "A camel treated by '264' method had no immunity when reinoculated". (2) *'643' combination.* Same as '264,' but the combination used at one-day intervals, the whole course lasting for 27 days. A single recovery was obtained by this method. (3) *'588' combination.* As in (1), but atoxyl inoculated subcutaneously in doses of 5 to 6 gm. and the entire combination administered 3 times with intervals of 3 and 7 days. Recovery was obtained in 1 out of a group of 5 animals treated. (4) *'436' combination.* As in (3), but atoxyl in doses of 5 to 7·5 gm. and the entire combination administered 4 times. Leese admits likelihood of poisoning by this method. (5) *'957' combination.* Sodium arsenate administered before atoxyl to increase effects of arsenic but possibilities of arsenic poisoning. Tartar emetic used up to 3 gm., but maximum safe dose found to be 1·5 gm. (6) *'178' combination.* Fifty-five grains of sodium arsenate by mouth on first day, 5 gm. atoxyl subcutaneously on 2nd day, 1·5 gm. tartar emetic intravenously on 3rd day, the course being repeated after 3 days. One recovered out of 10. (7) *'203' combination.* As in (2), but tartar emetic 1·5 gm. The single animal treated by this combination did not relapse in 6 months. Leese states that 12 per cent of the total number of animals treated by these methods died of the irritant effect of arsenic, apart from those that died of nephritis. In a further series of experiments, Leese (1913) used soamin—a proprietary preparation—which, for all practical purposes, may be regarded as equivalent to atoxyl. He lays down the following schedule—the so called '668' combination—as being the best : 5 gm. soamin subcutaneously, 0·5 gm. tartar emetic intravenously and 0·6 gm. arsenious acid on the first, second and third days respectively; the course is to be repeated after 2, 4 and 8 days, increasing the dose of soamin to 6 gm., 6·5 gm. and 6·5-7·5 gm. respectively. Leese claims to have obtained 62 per cent cures. '668' is briefly described by him as '436' with the sodium arsenate *per os* replaced by arsenic intravenously.

Gaiger (1909) records having unsuccessfully treated 7 surra camels either with orpiment alone or with a combination of atoxyl and orpiment. He considers the combination 'too expensive to be practicable with camels.' He also failed to obtain recovery in any of a pack of fox hounds when treated with atoxyl given subcutaneously and quinine given by the mouth.

Referring to Holmes's success in the treatment of surra, Gaiger (1911) remarks that the results recorded by Holmes are of little practical value, since the cases treated by him were artificial ones. Gaiger himself treated by Holmes's method 25 natural cases of equine surra with atoxyl, orpiment, arsenious acid, sodium arsenite and Fowler's solution, but only 4 out of the 25 (16 per cent) were cured. He found that atoxyl combined with arsenious acid (in bolus) was ineffective and that the best form of treatment 'for the Punjab strain of trypanosomes' was a combination of atoxyl and orpiment. Gaiger's finding, however, is difficult to reconcile with Holmes's statement that, as already referred to, orpiment may be regarded as a substitute for arsenious acid. Gaiger recommends arsacetin in place of atoxyl as being cheaper; this drug is administered in a 2 per cent normal saline solution subcutaneously.

It would seem that the success of atoxyl treatment of surra is often largely determined by the length of time for which the treatment can be pursued. This is illustrated in a case of an equine successfully treated by Lishman (1911) at Sialkot (Punjab). The animal, which had swarming trypanosomes in its blood, was given a dose of 3 drachms of soamin in 3 parts of water at body temperature and this resulted in a sterilization of the circulating blood in the same evening. The animal was given arsenious acid in bolus at 1 gm. on the 4th, 6th, 8th and 10th days and at 1·5 gm. on the 12th and 14th days. The trypanosomes reappeared in the circulating blood on the 15th day when a dose of 2 drachms of soamin was injected and this once again resulted in a sterilization of the blood. From the 16th to the 22nd day arsenious acid was given in increasing doses up to 2·5 gm. The animal had no relapse during an observation period of 4½ months and the treatment was thus regarded by Lishman as having resulted in a complete cure.

The results recorded by Cross (1914) would appear to point to the conclusion that repeated and progressively increasing doses of certain drugs are likely to yield much better results than single large doses. Whether in his trials with arsenicals or with tartar emetic, Cross consistently adhered to this principle. In his experiments with soamin and arsenious acid in the treatment of camel surra, Cross tested the relative merits of 4 lines of treatment : (1) One dose of 100 c..c of a 5 per cent solution of soamin followed by 10 doses of arsenious acid at 1- day intervals, the latter progressively increasing from 0·4 to 2 gm. Cure was obtained in 12 out of 30 (40 per cent) animals treated, the post-treatment observation period varying from 95 to 281 days. (2) Increasing doses, as above, of arsenious acid only. Cure was obtained in 11 out of 20 (55 per cent) animals treated, the observation period varying from 122 to 277 days. (3) Combination of soamin, tartar emetic and aresnious acid, in doses of 100 c.c. of a 5-7 per cent solution, 0·5 gm. and doses of 0·5 to 1·2 gm. respectively. The entire course occupied 3 consecutive days and then it was repeated 3 times at intervals of 2 to 6 days. Cure was obtained in 14 out of 24 (58 per cent) animals treated, the observation period varying from 73 to 379 days. (4) Soamin and increasing doses of arsenious acid, as above, given alternately at 1-day intervals, but some were given arsenious acid alone after a few doses of soamin. Cure was obtained in 10 out of 15 (66 per cent) animals treated. Cross classified some of his trials in accordance with the presence or absence of trypanosomes in the circulating blood, but these particulars are not included here as being of doubtful practical value.

From a paper published by Cross in 1915 on the treatment of camel surra with atoxyl it would appear that the results compared favourably with those obtained by him with tartar emetic (*q.v.*), for he states that recovery was obtained by him in 54 out of 68 (79·41 per cent) animals treated either by arsenic alone or by arsenic and soamin. In a subsequent report, he (1916) claims to have used these methods with success in 129 out of 158 (81·64 per cent) cases of camel surra treated by him. It would, however, appear that, as observed by a number of workers, the extent of success that may be expected from the use of these drugs is, as already indicated in the Introduction, largely determined by the strain of parasites that it is being sought to deal with. Thus, Montgomery (1916) in British East Africa (Nairobi) records having found both atoxyl and soamin valueless) in the treatment of trypanosomiasis in general. In drawing conclusions in regard to the efficacy of a drug, individual idiosyncrasy has to be given due consideration, the importance of this being illustrated by the fact that, Hutchins (1918), working in Uganda, mentions having cured *T. pecorum* infection in a cow by the subcutaneous injection of 5 gm. doses of soamin spread over a period of 5 months.

Walker (1914) tested the efficacy of a combination of atoxyl and arsenious acid. as recommended by Holmes, for equine surra. Atoxyl was injected subcutaneously in a dose of 100 c.c. of a 4 per cent solution and the acid was given in the form of a bolus in doses of 1·5 to 2·5 gm. The treatment proved ineffective. In view of the success obtained by Holmes with this form of treatment. Walker considers his results 'most disappointing'.

Outside India, in the early days of chemotherapy of trypanosomiasis, atoxyl was much in favour with a number of workers. Thiroux and Teppaz (1908) made a special study of the efficacy of atoxyl for the forms of trypanosomiasis known as 'souma' and 'gambia' disease in horses. They used atoxyl in 5-gm. doses, in combination with orpiment in doses of 15 rising to 25 gm., given in bolus or electuary. The orpiment was given on the first day and atoxyl on the 3rd, the 2 drugs being thus continued on 10 alternate days, ending with atoxyl. The course was repeated after 8 days. All of a batch of 3 horses were successfully treated by this method. It is, however, worthy of note that recovery was also obtained in 2 horses treated with orpiment alone at 3-day intervals, a second course being given, as in the combined treatment, after 8 days, In a later communication, Thiroux and Teppaz (1909*b*) describe the condition of the treated horses as satisfactory. Thiroux and Teppaz (1909*c*) mention having treated successfully 5 other horses affected with '*souma*'. In these cases they used atoxyl in 5-gm. doses and the orpiment, as before, in bolus or electuary. The post-treatment observation period was very short, but the non-occurrence of auto-agglutination in the 5 animals was regarded by them as suggestive of recovery. As already mentioned,

Thiroux and **Teppaz** (1910*a*), referring to equine surra, state that horses bear atoxyl badly and that even races of horses differ in their tolerance for this drug. In support of this statement, they point out that the horses experimented upon by Holmes bore 4 times as much atoxyl as did Thiroux and Teppaz's. They found that a dose of 2 to 3 gm. was ineffective and that cure was obtained only in the few which bore 5-gm. doses. Thiroux and Teppaz record having obtained the best results with a combination of orpiment and tartar emetic. On the other hand, they (1910*b*) found that domedaries stood atoxyl better than horses and they suggest atoxyl alone or a combination of atoxyl and tartar emetic in the treatment of camel surra. The administration of 4-5-gm. atxoyl combined with orpiment in bolus was found by them to cause a temporary sterlization, while a total of 5 intravenous injections of tartar emetic alone in 1½-gm. doses, with an interval of 5 days, caused the disappearance of the parasites for 21 days. Camels were found by them to bear orpiment badly.

Fraser and **Symonds** (1908) in the Federated Malay States unsuccessfully treated with atoxyl a horse experimentally innoculated with surra, the drug having been given in 0·5-gm. doses nearly every day for 4 days. Death occurred in 2 other horses, 1 treated with atoxyl in doses of 0·5 to 3 gm. in combination with perchloride of mercury in doses of 0·5 to 0·15 gm. and the other with antimony in addition.

The treatment of '*jaffa*' '*el debab*' and '*el zombab*' (presumably forms of *T. evansi* infection) in camels in Egypt was attempted by Mason (1911) on Holmes's lines, namely, by a combination of atoxyl subcutaneously and orpiment and sodium arsenate by the mouth, the doses. being 2 gm., 4·7 gm. and 4 gm., respectively. The drugs were given in the following order : 1st day atoxyl, 3rd day orpiment, 5th day sodium arsenate, 7th day atoxyl, and so on. Recovery was obtained in 4 out of 9 camels treated by this method.

Curasson (1918*b*) in French Sudan, tested the efficacy of a combination of atoxyl and picric acid for '*souma*' (*T. cazalboui* infection) in horses, '*baleri*' (*T. dimorphon* or *T. pecaudi* infection) in cattle and '*m'bori*' (*T. evansi* infection) in camels. Atoxyl was given subcutaneously in 10 per cent solution at 4 gm. for the horse and 5 gm. for the ox and the camel. Picric acid was given in increasing doses of 2 to 10 gm. for the horse and 12 gm. for the ox and dromedary, in the form of balls made of gum arabic and meal, each ball containing 50 cgm. extract of opium. Four injections of atoxyl every 5 days and 4 daily doses of picric acid in the intervening period were given, the entire course being repeated after a week. No relapse occurred in any of the treated animals during an observation period of about 3 months. Curasson (1921) also used a combination of atoxyl and tartar emetic, apparently with success, in the treatment of *T. pecaudi* and *T. dimorphon* infections in equines. Orpiment was also used in combination with atoxyl or tartar emetic. According to him, the addition of orpiment prolongs the effect of the other drugs, and these combinations, as devised by Teppaz, 'restent classiques'.

Sergent, Sergent and Foley (1921) used a combination of atoxyl and tartar emetic successfully in the treatment of '*debab*' in 2 dromedraries and the method adopted by them is worthy of consideration in some detail. The tartar emetic was injected intravenously 3 times at weekly intervals in doses of 2 gm., while 3 days later, a dose of 3 gm. of atoxyl was injected subcutaneously. In a series of further tests on 6 animals, they used tartar emetic in doses of 1 gm. for 4 successive weeks with 4 cures and 2 failures. The 4 successful cases were treated with 4, 5, 6 and 7 series of intravenous tartar emetic injections involving a total of 17, 20, 24 and 28 gm., in 9, 9, 10 and 11 months respectively. From these results they conclude that an average monthly dose of 2·2 gm., during a period of 10 months, may prove efficacious. The 2 unsuccessful cases each received 10 series of tartar emetic injections involving a total of 38 and 39 gm. respectively during a period of 1 year, so that a monthly dose of 3·2 gm. for 12 months would be toxic and ineffective. It is noteworthy that in the 4 successful cases treatment was undertaken later than in the other two. This, according to the authors, is suggestive of the possibility that resistant strains of trypanosomes are produced when treatment is undertaken early. Between atoxyl and tartar emetic, Sergent *et al.* (1924*a*) regard the latter as distinctly superior, the curative dose of atoxyl being very near the toxic dose.

Adams (1935) records having unsuccessfully used a combination of atoxyl and arsenious acid in the treatment of *T. evansi* infection in bovines in Mauritius.

Bouffard (1912), in treating *T. cazalboui* infection in the ox, found that atoxyl cleared the circulation only for a few days. This was also the case with orpiment and arseno-phenylglycin.

Fabre (1929) found that, in bovines suffering from *T. vivax* infection, injections of atoxyl in combination with orpiment by the mouth brought about recovery to a sufficient extent to enable them to return to work.

A combination of atoxyl, tryparosan and tartar emetic is claimed by Van den Branden (1919) to have been used with success in the treatment of *T. congolense* infection in cattle. The first was given by the mouth in doses of 16-17 gm., the second in doses of 0·26 gm. per kilo., also by the mouth, and the third on 5 successive days, intramuscularly, in a 2 per cent solution.

The experience of a vast majority of workers would appear to have been that, when used alone, atoxyl was altogether ineffective against trypanosomiasis, whether under field or laboratory conditions, but that in combination with tartar emetic it acquires striking therapeutic properties. This fact was well demonstrated by Van Saceghem (1915) in his work on the treatment of guinea-pigs infected with *T. brucei* and *T. congolense*, the combination having been found by him 'donner de trēs bons resultats'. Possibly, the therapeutic effect of the combined treatment is one of synergistic action. The mechanism of the effect of such combination would appear to merit further exploration, for Nattan-Larrier and Lepine (1928) found that the therapeutic effect of atexyl in experimental trypanosomiasis was reinforced when tetanus antitoxin was added to it, although the latter by itself had no action.

Atoxyl would appear to have been seldom tested in regard to its efficacy for dourine. Watson (1912) found the drug unsatisfactory in the treatment of natural dourine in Canada. Yakimoff (1915) has published a tabular statement summarizing the results of treating cases of dourine during a period of about 3 years. He claims to have obtained 'resultats excellents' with this drug. Uhlenhuth and Woithe (1908) state having had to use large doses of atoxyl in treating rabbits experimentally infected with dourine, this, according to them, having been due to the existence of an atoxyl-tolerance in rabbits and also to the fact that dourine assumes a chronic and, therefore, resistant form in these animals.

It is of interest that Beck (1910) found atoxyl highly effective in the treatment of rats and mice infected with *T. gambiense*; some of the treated rats lived over a year, although they became less resistant to re-infection. He states having successfully treated mice with a combination of Loeffler and Ruhs solution subcutaneously and atoxyl intraperitoneally. The best treatment for infected mice was found by him to be a combination of atoxyl and antimony. As a matter of fact, most of the early workers would appear to have advocated the use of atoxyl with some other durg, although why this shou have been so is difficult to understand in view of the success reported by many later workers from simple atoxylization in the treatment of human trypanosomiasis. Hams (1910), another early worker, likewise records having succeeded in curing each of a batch of 12 nagana-infected guinea-pigs by using a combination of Loeffler and Ruhs 'neu' solution in combination with atoxyl. He, however, found this combination valueless for horses.

Cases of recovery by the use of atoxyl alone have been occasionally recorded by early workers. Thus, Helm (1911) records the case of a dog infected with *T. brucei* in which a subcutaneous injection of 0·5 gm. of atoxyl effected a cure. A horse likewise was cured of *T. brucei* infection as a result of an intravenous injection of 8 gm. of the drug. Both animals had proved resistant to quinine therapy.

Friedberger (1908) tested the efficacy of atoxyl when administered in combination with thioglycollic acid (sulphydro-acetic acid, CH2 (SH), COOH) to white rats infected with nagana, but poisoning resulted It is possible that the combination causes an alteration in the arsenic group consequent on the reduction of atoxyl in the animal organism, thereby greatly increasing the toxicity of the drug—a possibility suggested by Blumenthal (1908).

Although arsenical preparations almost completely went out of use on the discovery of suramin in about 1921, Howard (1926) in India revived the use of atoxyl and crude arsenic in the treatment of equine surra. Two batches of diseased horses of 5 each were subjected to treatment with these two arsenicals as follows: (1) *Batch A*. Each given, on 3 successive days, 1,000 c.c. of 1 per cent solution of arsenious acid, 100 c.c. of 4 per cent solution of atoxyl intravenously and 1·5 gm. crude arsenic in bolus; this course was repeated every 3 days from 24. X to 20. XI; cure was obtained in 2 out of the 5. (2) *Batch B.* Atoxyl and crude arsenic given alternately at 1-day intervals, the treatment being continued for the same period as in Batch A: 2 recovered, but 1 showed inco-ordination. Recovery was, therefore, obtained in 4 (40 per cent) out of the total of 10 animals treated.

As a general principle for the protection of equines against trypanosomiasis in fly areas in the Cameroons, Dauzats (1928) recommended an intravenous injection of atoxyl every 10 days.

CHAPTER V

Arsenicals—contd.

TRYPARSAMIDE

IT is remarkable that tryparsamide, which, as a remedy for sleeping sickness, has ranked as a close second to suramin has only been infrequenty used in the treatment of animal trypanosomiasis. One reason for this would appear to be that it is the view of many—particularly French—workers that unlike other chemotherapeutic drugs, tryparsamide is generally ineffective in the early stages of human trypanosomiasis and that it shows itself at its best when the disease has advanced to an extent involving the cerebrospinal system, so that monetary consideration due to uncertainty of results has led many a veterinary worker to avoid the use of tryparsamide in the treatment of animal trypanosomiasis. The drug doubtless merits more attention in veterinary practice than it has hitherto received. In any case, the phenomenal success with which tryparsamide is claimed to have been used in the treatment of sleeping sickness, as will be seen presently, warrants a brief survey of the present position in regard to the use of this drug.

The outstanding advantage of tryparsamide over atoxyl, as pointed out by Chesterman (1923), is that the arsenic in this drug is held in stable pentavalent combination and not, as in atoxyl, in trivalent combination. The circumstances immediately preceding the discovery of this drug are briefly as follows. Pentavalent arsenicals being superior to trivalent in that they are both stable and soluble in water, Jacobs *et al.* (1919) investigated the possibility of synthesizing new types of organic trypanocidal compounds that will contain arsenic in the pentavalent form. Their-work resulted in the discovery of what came to be designated as tryparsamide or N–Phenylglycineamide-p-arsenic acid.

Brown *et al.* (1919*a*), from their studies on the action of tryparsamide on spirochaete and trypanosome infections in small animals, concluded that the drug could be safely administered in doses approximately equivalent to the lethal dose. An important finding made by them (1919*a*), and one that should prove of value in assessing chemotherapeutic drugs for practical use under field conditions is that, in infections due to *T. brucei*, *T. gambiense* *T. equinum*, *T. equiperdum* and *T. evansi*, the acute septicaemic forms without other clinical symptoms, occur in mice and rats, while the chronic tissue-invading forms, characterized by oedematous swellings, inappetence, etc. occur in rabbits. In *T. brucei* infection, the curative dose was found by them to be 0·2 to 0·3 gm. per kilo. for mice and rats and that for guinea-pigs 0·15 gm. per kilo. In rabbits infected with *T. brucei*, the curative dose was between 0·2 and 0·3 gm. per kilo., the chemotherapeutic index being between 3 : 1 and 2 : 1. Cure was obtained in 25 out of 29 infected rabbits, with similar results in those infected with *T. gambiense*.

Like other arsenicals, tryparsamide readily gives rise to strains resistant to it. It is worthy of note that, according to Launoy *et al.* (1936), tryparsamide-resistant strains of *T. annamense* in mice are more resistant than normal strains to trypaflavin and suramin. Launoy and Prieur (1935), from their observations on mice infected with *T. brucei*, have worked out some toxicity figures for tryparsamide, numerous samples of which were obtained by them from America, France and Belgium. They found that 50 per cent of mice weighing 20 gm. survived for 10 days after receiving a dose of 0·09 gm. of tryparsamide, while mice that had received 16 gm. of the drug remained relapse-free for 20 days; the co-efficient of trypanocidal activity of the drug was between 1 : 5 and 1 : 6·5.

A notable instance of the successful use of tryparsamide in the treatment of animal trypanosomiasis is recorded by Smillie (1923). He employed the drug intravenously in doses of 5 to 8 gm. each in 24 horses infected with mal de caderas. They were in good condition during a post-treatment observation period of 9 months, while all of a group of 5 controls died. It is noteworthy that Smillie considers the drug as of doubtful value when nervous involvement has taken place, for this view is contrary to the experience of some workers who have employed tryparsamide in the treatment of human trypanosomiasis.

Hornby (1925) states that tryparsamide is not only ineffective against *T. congolense* infection in bovines, but that it even makes the blood 'extraordinarily' favourable for the multiplication of the parasites. He records consistent failures to obtain recovery when the drug was used in the following dosages: (1) 100 c.c. of a 10 per cent solution; (2) 20 gm. in 80 c.c. water; (3) 45 gm. in 4 10-gm. doses on successive days followed by a 5-gm. dose; and (4) 50 gm. in 5 10-gm. doses.

A similar experience is recorded by Van Saceghem (1925*a*), who used tryparsamide intravenously in a 40 per cent solution in 2 animals infected with *T. congolense*. They received 40 gm. of the drug, one in 3 doses spread over 7 days and the other in 2 doses at a 5-day interval. Both died of what appeared to be drng poisoning, without any sterilization of the circulating blood having been effected. From further experience in the use of tryparsamide in the treatment of *T. congolense* and *T. cazalboui* infections, he (1926) categorically declared that the drug was valueless and that it failed to sterilize even when employed in the toxic dose of 0·066 gm. per kilo.

In India, likewise, Edwards (1926) records having found tryparsamide 'entirely disappointing' as a remedy for experimental surra in equines. He further states that it is rapidly excreted and that prophylactically it is even inferior to tartar emetic.

In the treatment of human trypanosomiasis, tryparsamide ranks with atoxyl, tartar emetic and suramin as a drug extensively used by a considerable section of workers. Trolli (1929) even declares, from his experience in the treatment of sleeping sickness in Belgian Congo, that it is unsurpassed in efficacy by any other drug introduced in the field of chemotherapy. The drug has, as already remarked, received little attention from veterinary workers and the records of its use in human trypanosomiasis are, therefore, only briefly considered here. As found by numerous workers (e.g. Walravens, 1926 and others), the drug shares with atoxyl the disadvantage that it frequently causes ocular damage when it is used over a long period.

Lefrou and Ouzilleau (1922*a*) do not consider tryparsamide superior to atoxyl and prefer its subcutaneous to intravenous injection. Most wokrers, however, use the drug intravenously, while Van den Branden and Van Hoof (1923) record having injected it also by the intramuscular route. Lefrou and Ouzilleau (1922*b*) found that the subcutaneous administration of 2 gm. of the drug resulted in sterilization of the blood for 1 year and of 1 gm. for 84 hours. These durations would appear to be the maximum as yet recorded for any other drug.

Chesterman (1924*a*) used the drug intravenoulsy in the treatment of 40 cases of human trypanosomiasis of which (*a*) 24 had received no previous treatment, (*b*) 10 had been previously treated with atoxyl, tartar emetic, etc. and (*c*) 6 had been intrathecally treated with medicated serum. Five of category (*c*) survived the intrathecal injection and of these 2 relapsed and died shortly after. Of the remaining patients, as many as 15 are stated by him to have maintained good health when examined after a period of 2 years. These patients were given a single course of tryparsamide totalling 10 to 28 gm. Chesterman states that he is convinced that the curative power of tryparsamide in nervous infections is superior to that of suramin, which latter is mentioned by him to cost $ 15 for 10 gm. or 10 times as expensive for a single course. Chesterman (1925) lays down the maximum single dose as 4 gm., this dose, if tolerated, being continued, in advanced cases, at weekly or fortnightly intervals until a total of 10 injections have been given. He recommends another short course after an interval of 6 months.

Chesterman *et al.* (1927) record having found tryparsamide also efficacious when it in given by the mouth in gradually increasing doses up to a maximum of 0·15 gm. per kilo.

In a paper published in 1932, Chesterman describes the results of tryparsamide treatment in 17 first-stage and 25 second-stage patients. From his results, Chesterman concludes that, for patients in the first category, a suitable course of treatment is a total of 8 weekly injections of 0·045 gm. per kilo. and for those in the other category, 0·16 per kilo. For advanced cases he recommends 2 or 3 courses of large doses, of the drug at 5-day intervals, with 2 weeks' rest after each course. The dosages represent 'a modification of the opinions expressed in previous publications by the author, when less drastic treatment was advocated and more prolonged courses deemed necessary.'

The superiority of tryparsamide over atoxyl according to Laigret (1926) is that, unlike atoxyl, it is effective in the more or less advanced stages of sleeping sickness, although in the early stages both are of equal value. He calls tryparsamide 'le medicament de choix' for human trypanasomiasis. Ledentu (1927) records 40 recoveries out of a total of 60 patients treated with tryparsamide in the advanced stages. He appears to have used a total of about 25 gm. of the drug for patients of average weight of 50 kilo. He claims to have obtained cures to the extent of 62·5 per cent in the second stage of the disease. In another paper published by him in collaboration with Vaucel, he (1927*c*) records having obtained 23 per cent cures in the terminal stages of sleeping sickness—a claim which has to be regarded as unique. The commencing dose recommended by these workers (1927*d*) is 0·035, progressively rising to 0·09 gm. per kilo., given weekly by the intravenous route.

Van den Branden (1925), from his experience in the treatment of 115 cases of sleeping sickness, recomends the use of the drug in weekly doses of 2 gm. until a total of 20 to 80 gm. has been given. He (1926*a*) apparently regards tryparsamide as a specific for human trypanosomiasis and attributes failures to concurrent syphilis or previous treatment with other arsenicals. King (1926) also ascribes failures to earlier use of arsenicals. The schedule of dosage adopted by him was 1 or 2 series of 10 weekly injections of the drug at 3 gm. per 50 to 60 kilo.

Van den Branden (127*a*) prescribes weekly doses of 2 to 3 gm. for adults and 0·5 to 1 gm. for children, with a total of 20 to 40 gm. in the first stage of the disease and 50 to 100 gm. for chronic cases. He (1927*b*) claims to have obtained 25 cures out of a total of 61 in the latter category. Lopes (127) also records having cured 12 out of 25 chronic cases with a total of 23 to 118 gm. of tryparasmide in each case. Jamot (1929) recommends a dose of 4·5 gm. per kilo. given weekly by the intravenous route. One or more courses are prescribed by him for chronic cases.

Corson (1928) cites the interesting instance of a medical worker who contracted trypanosmiasis in the first week of May, with the appearance of symptoms about a fortnight later Five 1 to 2-gm. doses of suramin administered at intervals varying from 2 to 5 days resulted in a cure lasting only for a fortnight, but 4 short courses of tryparsamide at monthly intervals resulted in complete recovery. The histroy of this case is contrary to what one would have expected, inasmuch as suramin is acclaimed as a drug of choice for use in the early stages, as tryparsamide in the advanced stages of trypanosomiasis.

Corson (1931*a*) found tryparsamide ineffective in 9 cases of cerebrospinal involvement. In these instances, he used a combination of 1 gm. of suramin and 2 to 4 gm. of tryparsamide at weekly intervals, a total of as much as 50 gm. of the latter drug having been given by him in one instance. He writes: 'The observations indicate that neither Bayer 205 nor tryparsamide can be regarded as likely to cure advanced cases of Rhodesian sleeping sickness. Corson's findings are contrary to the claim made by Pearce (1921, 1929)—one of the accredited authorities on tryparsamide—that the drug has a sterilizing effect on the cerebrospinal fluid, with a decrease of about 60 to 90 per cent in the cellular content in 5 weeks.

Tryparsamide was used by Lauterberg (1929) in 29 advanced cases of sleeping sickness in French Equatorial Africa. From the results of his trials, he concludes that the drug is capable of effecting a recovery of 60 per cent in the most advanced cases, a total of 7 gm. having been found by him adequate for this purpose. The claim, however, is not borne out by the experience of later workers.

The drug is acclaimed by Carpenter (1929) as of remarkable efficacy when given in 8 weekly injections of 3 to 4 gm. Maclean (1929*a*) likewise, from the results of his work in the Tanganyika territory, regards tryparsamide as the only drug that holds out a prospect of recovery in advanced cases, although the long course of treatment with this drug makes it much less convenient to use than suramin. Johnson and Lloyd (1929) lay down 13 gm. as the total quantity of tryparsamide to be used for a full course of treatment.

Two separate lines of treatment are recommended by Vaucel (1929), one for the first and the other for the second stage. For the former, he recommends the employment of 'Fourneau 270 up to a maximum of 0·3 gm. per kilo. or large doses of atoxyl. For the latter, he lays down weekly doses of tryparsamide, progressively rising from 0·015 to 0·06 gm. per kilo until the cerebrospinal fluid returns to normal, the treatment being in a series of 7 injections with 45 days interval between each 2 series.

According to Leger (1930), treatment with tryparsamide should be continued over a long period, for even a total of 27 gm. given in 7 or 10 weekly doses often prove insufficient. It is worthy of note that, while a total of 30 to 40 gm. of the drug is regarded as necessary to obtain recoveries, Lauterberg (1930) claims to have been successful with only 8 weekly injections of 0·5 gm. each.

Sicé (1931) has given some figures to show the relative efficacy of the 3 arsenicals—atoxyl, tryparsamide and 'Fourneau 270'. In the first stage, patients treated with these 3 drugs relapsed to the extent of 15·9 per cent, 19·6 per cent and 3·7 per cent respectively, while the disease progressed to cerebrospinal involvement in 25 per cent, 3·9 per cent and 2·8 per cent, respectively. In second-stage patients treated with tryparsamide and 'Fourneau 270', relapses occurred in 4·6 per cent and 3·5 per cent respectively, while progressive evolution occurred in 13 per cent and 13·1 per cent respectively. From these figures, it would appear that 'Fourneau 270' is distinctly superior to the other two.

Most French workers would appear to have consistently used arsenicals in the treatment of sleeping sickness and have resorted to suramin in exceptional cases. Even for the first stage of the disease, Martin (1931) recommends either orsanine ('Fourneau 270') or atoxyl, the use of suramin being resorted to in cases of arsenic resistance or of relapse. According to him, tryparsamide should be used in the meningeal stage, with the addition of orsanine or atoxyl in the event of concurrent blood infection. The French worker Muraz (1931, 1932) lays down as the 'standard' treatment a course of 12 injections of orsanine or tryparsamide. As a matter of fact, quite a number of schedules have been drawn up from time to time by different workers as being 'standard' for the treatment of human trypanosomiasis. One such cure, given by Laurent (1932), consists of 15 doses of 2 gm. of tryparsamide administered at weekly intervals.

In a paper published by Vaucel (1931*a*) in collaboration with Boisseau, he categorically declares tryparsamide as altogether valueless in first-stage sleeping sickness, but states that it is the only drug which can be used with some prospect of recovery in the second stage. In another paper, Vaucel and Boisseau (1931 *b*) stress the desirability of prolonged treatment with tryparsamide at 0·4 gm. per kilo. The results of such treatment are tersely described by them as being either complete recovery or complete failure. Maclean (1931) records having obtained uniformly good results in the treatment of 450 cases of nervous involvement in human trypanosomiasis by a total dosage of 10 to 18 gm.

What would appear to be a rather drastic method of using tryparsamide has been suggested by Kellersberger (1933). According to him, the first course of this drug should comprise a total of 100 gm. administered in weekly doses of 0·045 gm. per kilo. with an interval of 1 to 3 months before the commencement of a second course.

A paradoxical observation recorded by Loré and Marty (1933) on the effect of tryparsamide is that, when given in the second stage of the disease, it is ineffective if it had been used in the first stage, but recovery usually takes place in the second stage if other arsenicals, such as atoxyl and novarsenobillon, but not tryparsamide, had been used in the first stage. Obviously, the phenomenon cannot be regarded as one of simple arsenic-fastness. Jamot and Vernon (1927) had already observed that the use of tryparsamide was not contra-indicated in patients previously treated with atoxyl. He had used a weekly dose of 4 cgm. per kilo. until a total of 10 injections had been given.

From a perusal of the records of treatment with tryparsamide it may be concluded that a large majority of workers, although placing great relance on this drug, are agreed that the most promising line of treatment is to use tryparsamide after a short course of treatment with suramin. According to Manson-Bahr (1232), the most effective treatment in relapses is to administer a total of 3 gm. of suramin in the first week and then a course of tryparsamide in tolersted doses.

Ellie (1934) noticed that tryparsamide had a greater predilection for the glands than for the circulating blood. He treated 829 cases with the drug at 6-day intervals, with 2-gm. doses—the initial dose being only 1 gm.—until a total of 13 injections had been given. On the completion of treatment, 15 patients were still positive and of these, 13 showed parasites in the blood and 2 in the glands only.

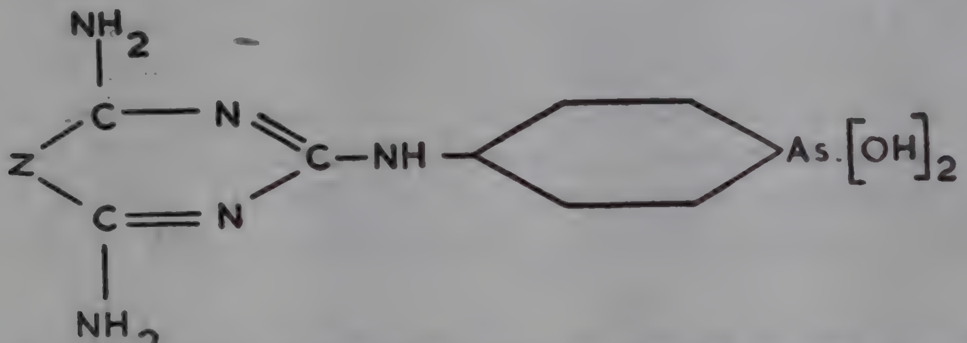
Amongst other workers who have dealt with the subject of tryparsamide in the treatment of human trypanosomiasis, mention may be made of: Van den Branden (1923); Letonturier *et al.* (1924); Morgan (1924); Blanchard and Laigret (1925); Laigret (1925); Abbatucci (1926); Lewis (1926); Schofield *et al.* (1926); Kellersberger (1927); Almeida (1928); Van Hoof (1928*a*, *b*); Kikuth (1929); Corson (1930); Corson (1931*b*); Saunders (1932); Sicé *et al.* (1933*a*, *b*); Pottier and Van den Branden (1935); Van Slype (1935); Lester (1938); Kopp and Solomon (1940); and Grant *et al.* (1944).

CHAPTER VI

Arsenicals—contd.

MELARSEN

MELARSEN, or triazine arsinic acid, or No. '4289,' or '2224 RP',—an aromatic arsenical —is a pentavalent compound and was prepared by Friedheim (1940, 1941), who gives its formula as 2, 4-diamino-6 (arsono-anilino) triazine with the following chemical constitution:



The drug does not appear to have been tested on clinical cases of animal trypanosomiasis but it has been used with outstanding success in laboratory trials and also in sleeping sickness. It has, therefore, been considered desirable to include a brief account of this drug in the present monograph. In the laboratory, the toxic dose of the drug was found to be 0·75 to 1 gm. per kilo., this dose having killed 10-20 per cent of the mice. The tolerated dose for dogs was 0·2 gm. per kilo. In human trypanosomiasis, sterilization was obtained within 24 hours after parenteral administration and within 3 days after oral administration. By the former method the drug was given in doses of 0·03-0·5 gm. per kilo. with a maximum of 2 gm. every 3 days until a total of 10 injections had been given. Of a total of 76 patients treated parenterally, 29 were regarded as clinically cured on re-examination after 2½ to 9 months. Thirteen were treated orally with '4289' and, although they were sterilized, some of them developed gastro-intestinal trouble.

The corresponding oxide of melarsen—known as melarsen oxide, or melox or '3177 RP'—is reported by Weinman (1946) to have been non-toxic for human subjects when it was used orally or intravenously at a dose of 0·1 mgm. per kilo. Nine early cases treated—apparently intravenously—with this drug did not relapse during an observation period of 6 months and were considered to have been cured. Some amelioration occurred also in advanced cases.

A brand of melarsen oxide, prepared by Parke, Davis & Co., was tested by Weinman and Franz (1945). It was given orally in a daily dose of 3 mgm. per kilo., for 5-8 days, to 12 patients, 8 of whom showed symptoms of cerebrospinal involvement; cure was reported in 11 of these cases. Equally good results were obtained in 8 patients when the drug was given by the intravenous route for 7 days at 0·1 mgm. per kilo. Three other patients with nervous symptoms were similarly treated, but the treatment was successful only in 1.

Van Hoof (1947), however, states that he found melarsen oxide as too toxic for human subjects. According to Nodenot (1947), melarsen is a mediocre remedy for humn trypanosomiasis in the second stage and is inferior to melarsen oxide.

A series of very important observations was published by Rollo *et al.* (1949) on the trivalent oxide of melarsen and its antimony analogue, sodium-p-melaminylphenyl stibonate

(MSb_3) and also on the pentavalent melarsen and its antimony analogue MSb. As the result of their studies on the effect of these drugs on *T.gambiense* in mice, Rollo and his collaborators concluded that melarsen possessed a higher therapeutic index than tryparsamide, while MSb was still better in this respect. Melarsen oxide and MSb_3 showed a higher index than mapharside (oxophenarsine hydrochloride), or butarsen (y-(*p*-arseno-phenyl) butyric acid, or '70A', or 'para-arseno'). When used in rabbits, both melarsen and MSb showed a better therapeutic effect than tryparsamide. MSb was found remarkably powerful and 'long-lasting' in effect as a prophylactic agent, for a single intraperitoneal injection of the drug protected mice against *T.rhodesiense* for 41 weeks, against 10 weeks in the case of suramin and about 2 weeks in the case of pentamidine, while antrycide salts protect cattle against *T.congolense* for not more than 6 months. Rollo and his collaborators make a special reference to the fact that this protection was obtained with the polymerized salt, and not the crystalline form, of MSb. It would appear that the protective power of MSb is to be largely attributed the slow excretion of the drug as compared with that of other aromatic antimonials. Williamson (1949) has shown that after intraperitoneal injection of comparable doses of MSb and stibenyl, 54 per cent of the antimony in MSb is excreted in 5 days, as against 96 per cent of the antimony in stibenyl.

The laboratory indications concerning the remarkable prophylactic power of MSb were followed up by an extensive series of tests conducted with this drug on human subjects in French West Africa. A series of concurrent tests was also made with pentamidine and lomidine. The results are summarized in Table I (Le Rouzic, 1949).

TABLE I

Results of prophylactic use of pentamidine, lomidine and MSb in sleeping sickness.

Drug	Total number of persons [illegible] 1946-48	Found infected during a follow up of 5-12 months
Pentamidine	637	1
Lomidine	1 146	7
MSb	794	

The results obtained by 3 observers independently from the use of MSb are given in Table II.

TABLE II

Results of three observations on the use of MSb

Observer	No. of people treated	Time of follow up	Number examined	Number found infected
		Months		
1	364	4-5	364	0
2	50	8	50	0
3	380	4	60	0

In view of the spectacular results obtained by the above workers with MSb, some work would appear to be distinctly warranted to test the value of this drug as a prophylactic agent against surra in India.

Since the discovery of the drug 2, 3-dimercaptopropanol, commonly known as 'BAL' (British Anti-Lewisite), workers have utilized its detoxicating properties by combining it with trivalent derivatives of arsenical and antimony compounds so as to render such derivatives considerably less toxic. Citing the instance of tryparsamide, Friedheim (1940*b*) explains that this relatively non-trypanocidal pentavalent arsenical becomes trypanocidal only when it is reduced to the trivalent form which, however, is both toxic and unstable. These disadvantages were overcome by converting it into the form—apparently by treatment with 'BAL' —called by him 'T.P.B.' which is stated by Friedheim to contain 21·6 per cent trivalent arsenic. He claims to have obtained sterilization by single injections of 'T.P.B.' in 10 second-stage cases of *T.gambiense* infection; 5 of these were given the drug by the oral and 5 by the intravenous route. Friedheim succeeded in reducing the toxicity of the trivalent melarsen oxide by combining it with 'BAL', the resultant product being known as 'melarsen BAL' or in the abbreviated form, as 'MelB'. According to Friedheim, such detoxicated products not only possess superior trypanocidal power but also exercise a favourable action on the cerebrospinal fluid. The trivalent antimonial analogue of melarsen oxide, MSbIII, has also been similarly combined with 'BAL,' the product of this combination being known as 'MSbB'. Similar products by combining with 'BAL' have been claimed to have been obtained from aromatic arsenicals, such as carbarsone (4-carbamidophenylarsonic acid) and stovarsol, these products being obtained from tryparsamide and called 'C.B.B.' and 'S.T.B.' respectively. Le Rouzic mentions 'detox', representing detoxified tartar emetic. Concerning 'MelB', Friedheim (1949*a*) writes that he tested this drug on 50 advanced cases of sleeping sickness, including 20 showing trypanosomes in the cerebrospinal fluid. The drug was administered intravenously in doses of 2 to 4 gm. per kilo., a total of 3 to 14 injections being given either in 1 or 2 series. The treatment resulted in sterilization of the lymphatico-blood system within 24 hours, while 9 of the patients, who had shown trypanosomes in the cerebrospinal fluid, were found to be free from these when examined one week after the treatment. The treatment recommended by Friedheim for all stages of human trypanosomiasis is 4 intravenous injections of the drug at 3·6 mgm. per kilo. followed after one week by another 4 similar injections.

Friedheim (1948) used melarsen oxide with highly satisfactory results in human trypanosomiasis in French Guinea, and he states that the drug eliminates trypanosomes from the cerebrospinal fluid after treatment of only one week's duration. The course consisted of a series of intravenous injections for 7 days at 1·5 mgm. per kilo., followed, after an interval of 1 month, with another similar series of injections. The drug did not produce any toxic symptoms. Five advanced cases were followed up after treatment and the cell count and protein content of the cerebrospinal fluid in these patients returned to normal in from 3 to 9 months. Friedheim points out that the two-fold superiority of melarsen oxide over tryparsamide is that it is effective both in the early and late stages, while tryparsamide is particularly effective in cases of cerebrospinal involvement; secondly, the treatment with melarsen oxide occupies only 6 weeks, against a period of 7 months required by treatment with tryparsamide.

The efficacy of melarsen and melarsen oxide, when given orally to human patients, was tested by Trinquier and Pellissier (1948) and they concluded that the drug was effective in the lymphatico-blood stage of the disease but was of little value when cerebrospinal involvement had occurred and that it 'was dangerous for patients in the meningo-encephalitic stage.' The dosages recommended by them are: (1) *Melarsen.* To begin with 20 mgm. per kilo., rising by 10 mgm. per kilo. daily till the 5th day and thereafter this dose, that is, 60 mgm. per kilo., for another 9 days, subject to a ceiling dose of 3 gm. (2) *Melarsen oxide* (*flat doses*). First day, 10 mgm.; 2nd to 5th day, 20 mgm.; 6th to 14th day, 30 mgm.

Le Rouzic and Lapeyssonie (1948) have briefly described the relative values of melarsen and its various derivatives as follows : (1) *Melarsen.* Tested on 22 advanced cases of sleeping sickness in French West Africa ; the cerebrospinal fluid returned to normal in 8 and improved in 6. According to these workers, the superiority of melarsen over tryparsamide consists in the facts that the latter either restores the cerebrospinal fluid immediately to normal or has no action on it at all and that, while melarsen exerts a favourable action on both the lympatico-blood stage and the cerebrospinal fluid, tryparsamide acts particularly on the latter. (2) *Melarsen oxide and MelB.* Superior to melarsen by reason of their efficacy in the second stage and the shorter course of treatment which they entail. (3) *MSbV. MSb III, MSb.* Effective in early stages but uncertain in the second stage.

Le Rouzic (1949) has given some figures to show the relative extent of success with these drugs in the treatment of human trypanosomiasis. (A) *First stage of disease.* (*i*) T.P.B., 2 cases and both successes. (*ii*) MSb V, 109 cases. with 32 per cent failures. (*iii*) Combined MSb and MSbB, 90 cases, with 20 per cent failures, (*iv*) Detox, 10 cases with 1 failure. (B) *First and second stages of disease (not clearly differentiated*). (*i*) Melox, 40 cases, with 20 per cent failures. (*ii*) S.T.B., 6 cases, with 3 failures. (C) *Second stage.* (*i*) Melox, 33 cases, with 27 per cent failures. (*ii*) T.P.B., 10 cases, with 2 failures. (*iii*) C.B.B., 6 cases with 5 successes and death. (iv) MSbV, 15 cases with 20 per cent failures. (*v*) Combined MSb and MSbB, 16 cases, with 19 per cent failures. (*vi*) Detox, 5 cases, with 2 failures. With MelB:(*a*) *First stage.* 40 cases, with 12·5 per cent failures. (*b*) *Second stage.* 174 cases, with 5 per cent failures. Le Rouzic, on the authority of Lotte, mentions MelB as exerting an energetic action on blood, glands and cerebrospinal fluid. It is also claimed to be harmless for the kidneys and the optic nerve and to generate a sensation of well-being in the patient. It is, however, stated to be more toxic than lomidine. The French Commission, consisting of Schneider, Marchalant, Lotte, Bouisson and Diallo (1949), reported the following results with MelB, in French West Africa, in the treatment of human trypanosomiasis. (A) *First stage.* 41 cases, with 2·5 per cent failures. (B) *Second stage.* 135 cases, with 10 per cent failures. (C) *Chronic stage.* 37 cases, with 12 per cent failures. These results led the Commission to regard the drug as possessing remarkable remedial properties.

Ceccaldi (1950), during his directorship of the Pasteur Institute at Brazzaville, published a large series of detailed observations on the value of melarsen and the related compounds in the treatment of sleeping sickness. Concerning melarsen oxide—also known as '3177 RP'—Ceccaldi *et al.* (1949*a*) mention having used the drug intravenously in human trypanosomiasis in doses of 0·1 mgm. per kilo. on the first day, 0·2 mgm. on the second day and 0·3 mg. daily on the next 5 days; the post-treatment observation period ranged from 3 to 20 months. The conclusion arrived at by these workers was that the drug was effective in the earliest stages, but that a second dose of the drug had no favourable action on the cerebrospinal fluid even when slightest changes in it had set in. This view was contended by Schneider (cited by Ceccaldi *et al.*, 1949 *a*), who pointed out that Friedheim himself had used the drug successfully in this stage in a dose of 1·5 mgm. daily for 7 days, followed by a second similar course after an interval of 1 month. Friedheim's method was tested by Ceccaldi *et al.* (1949*b*) on 10 patients in the meningo-encephalitic stage, the post-treatment observation period in these cases being only 3 months. The results were as follows. Seven of the patients stood the treatment well, but 3 developed severe toxic symptoms, which were mitigated in two by the injection of disodium thiomalate, but in the third the symptoms progressed to a fatal issue. Although there was a distinct improvement in the nine that survived, these workers consider that the drug is too toxic to lend itself for use with safety. Ceccaldi *et al.* (1950) record having obtained satisfactory results with MelB when, as recommended by Friedheim, it was administered intravenously in a dose of 3·6 mgm. per kilo. daily for 4 days, followed by a second course after 1 week. Twenty-one patients were treated and 20 of these were in an advanced stage of the disease. Death occurred only in 3 of the patients, this being attributed to the toxic action of the drug. The treatment was found to cause a quick restoration of the cerebrospinal fluid to its normal condition although this improvement was not always maintained over long periods. They refer to MelB as a 'medicament interessant', but consider its posology, as recommended by Friedheim, much too elevated ('de loin trop elevée').

MelarsenB was used by Ferreira *et al.* (1950) in Portuguese Guinea on 107 cases of human trypanosomiasis. Of these, 5 were in the first, 23 in the second and 79 in the third stage. The dosage used was that recommended by Friedheim (*supra*), a single course being given to 38 and a second course to the other 69 after one week. Twenty of the patients in the second stage were examined after 6 months, and of these, 16 were found improved and 4 worse. Sixty-seven of the third-stage patients were examined periodically for 1 year; of these, 59 were found improved, 2 worse and 2 unchanged, while the condition of the remaining 4 progressed to a fatal issue.

A series of trials was carried out by Duggan and Hutchinson (1951) on 700 cases of human trypanosomiasis in Northern Nigeria with melarsen compounds. These workers state that, when given intravenously at 5-day intervals, 8 injections of melarsen at a dose of 15 mgm. per kilo. were at least as effective as 12 injections of 3-gm. doses of tryparsamide. When administered intravenously on 7 successive days, melarsen oxide, at 0·1 mgm. per kilo., yielded results comparable to those obtained with mapharsen at 0·4 mg. per kilo. and is 'undoubtedly effective in a high proportion of intermediate and advanced cases.' Melarsen oxide, according to them, proved highly toxic. Cure was obtained in all of a small number of cases treated with MelB at the recommended dose of 3·6 to 4 mgm. per kilo for 4 days; this drug proved more toxic than melarsen or melarsen oxide. It is worthy of note that, as for the claim made by certain workers that MelB exerts a favourable action on the cerebrospinal fluid, Monnet and Baylet (1951) found that it penetrated this region only to a slight extent after intravenous injection.

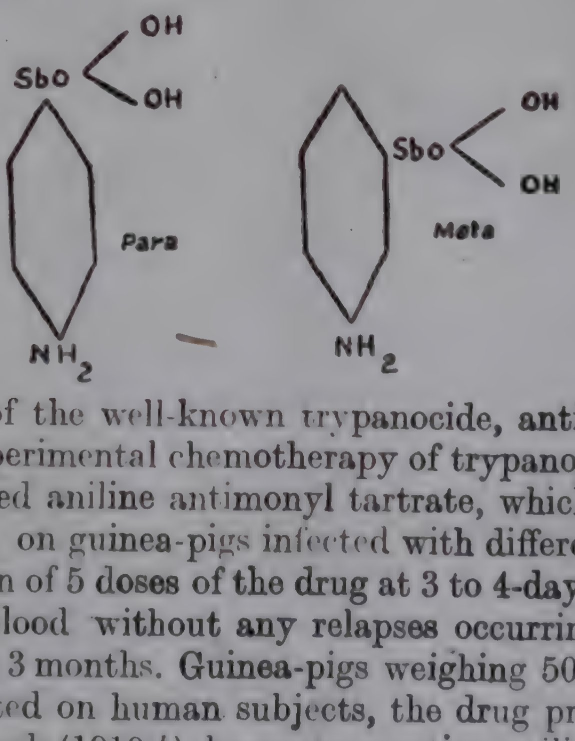
CHAPTER VII

Antimonials

INTRODUCTORY

A PERUSAL of the history of antimonials that have been brought into the field of chemotherapy of trypanosomiasis shows that quite a legion of these in various forms have been used in the treatment of this condition, such as tartar emetic, anthiomaline, stibophen (fouadin), stibsol, stibosan ('Heyden 471'), solustibosan, sodium antimony gluconate (trivalent), sodium mannitol antimoniate, neostam, neostibosan, ureastibamine, and stibacetin. To this list may be added 'Heyden 661'—stated to be an antimony complex salt ('dreiwertiges Komplex gebundenes Antimon') which cured dourine-infected mice in 2-mgm. doses (Uhlenhuth *et al.*, 1924), the series of Dn and antimosan. Of these, tartar emetic and antimosan will be dealt with separately. According to the experience of Laveran (1910), *T. dimorphon* and *T. congolense* respond better to antimony salts than to atoxyl.

The work of Breinl and Nierenstein (1909*b*) throws some interesting light on the relationship of the therapeutic effect of antimonials with their chemical constitution. In an attempt to obtain an organic compound of antimony analogous to atoxyl, they prepared and studied the *para* and *meta* compounds of amino-phenyl-stibonic acid. They found these compounds to be powerful trypanocides but less active than sodium antimonyl tartrate, the *para* being superior to the *meta*, as had been stated by Ehrlich to be the case with the corresponding arsenic compounds. The graphic formulae of these two classes of compounds are given by them as follows:



Several modifications of the well-known trypanocide, antimonyl tartrate, have been brought into the field of experimental chemotherapy of trypanosomiasis from time to time. Thus, Laveran (1909*a*) tested aniline antimonyl tartrate, which contained 30·22 per cent antimony, intramuscularly, on guinea-pigs infected with different species of trypanosomes including *T. evansi*. Injection of 5 doses of the drug at 3 to 4-day intervals resulted in sterilization of the circulating blood without any relapses occurring during a post-treatment observation period of about 3 months. Guinea-pigs weighing 500 gm. bore satisfactorily a dose of 2·3 cgm. When tested on human subjects, the drug proved less toxic than tartar emetic. Martin and Ringenbach (1910 *b*), however, mention aniline emetic as having proved valueless against sleeping sickness. Laveran (1910*c*) also prepared crystals of antimonyl tartrate of arsenic and aniline from a mixture of aniline antimonyl tartrate and aniline arsenyl tartrate. Emetic of arsenic and antimony showed 'grande efficacite' and proved superior to 'emetique arsenical et aniline'. When injected intramuscularly in a 2 per cent aqueous solution into guinea-pigs infected with *T. evansi*, *T. congolense*, *T. dimorphon* and *T. gambiense*, cure was obtained after a single course of the treatment.

Plimmer (cited by Ranken, 1913) introduced the use, by the intravenous route, of finely powdered precipitated metallic antimony for the treatment of trypanosomiasis, the course consisting of 5 1-gm. doses at 4 day intervals, followed after 6 weeks by a course of 3 doses extending over 9 days.

Thomson and Cushny (1910) mention ethylantimonyl tartrate as having been found a very effective trypanocide when tested against experimental trypanosomiasis in rats; sterilization was effected in 2 hours and recovery was obtained in 7 out of 13 rats treated.

Rowntree and Abel (1910) tested triamide of antimony thioglycollic acid and sodium antimony thioglycollate against experimental trypanosomiasis in white rats, dogs, a donkey and rabbits; the two drugs were found to be as effective as any other antimonial previously used. In a paper published by the same authors in 1911, they confirm their findings and also refer to the low toxicity and non-irritant properties of these drugs, while the therapeutic results in the treatment of experimental trypanosomiasis are described by them as having been 'excellent'.

Kolle, Hartoch, Rothermundt and Schurmann (1913) claim to have effected a permanent disappearance ('Dauersteriliserung') of nagana trypanosomes from the circulating blood of mice when the latter were injected intramuscularly with a 1-gm. dose of powdered metallic antimony suspended in 30 per cent concentration in oil. These workers would also appear to have been the earliest to make the finding that pentavalent compounds of antimony are less toxic (see, however, Hartoch, Rothermundt and Schurmann, 1913, *infra*) and also therapeutically less active than the trivalent compounds, the most important representative of these latter being the trioxide, designated by them 'trixidin'–a drug which is insoluble in water and is slowly absorbed. For nagana in mice, an effective dose was found to be 1 mgm. per 10 gm. body weight. Hartoch, Rothermundt and Schurmann (1913) referred to the fact that trixidin is non-toxic for small animals, for, as already mentioned all other known trivalent compounds of antimony are poisonous for these animals. Kolle, Hartoch and Schurmann (1914), as a result of further experiments with trixidin, concluded that the drug, although curative for dourine in rabbits and guinea-pigs, caused abscess formation when injected intramuscularly to these animals, but this sequela could be avoided by injecting the drug in a colloidal form with salt solution. They mention having obtained sterilization for at least 2 months from the use of trixidin in bovine trypanosomiasis.

Lurz (1914) found that, when injected as a 30 per cent emulsion in oil, trixidin was invariably effective in experimental trypanosomiasis in small animals, while Horst (1918) observed that trixidin was even more effective than tartar emetic in the treatment of nagana and dourine in rabbits and guinea-pigs. Lurz (1914), as also Hellen (1914) and Van den Branden (1920), mentions trixidin as being ineffective against human trypanosomiasis. Hellen states that the injection of this drug caused high fever and abscess formation necessitating surgical interference ('hohen Fieber zur Abszessbildung'). Daniels and Newham (1916), however, claim to have cured a case of human trypanosomiasis by the subcutaneous injection of Martindale's injectio antiomonii oxidi (equivalent to 1/20th grain tartar emetic), followed a week later by what would appear to be antimony trioxide. It is possible that in this instance recovery was really due to Martindale's solution, in view of a case of sleeping sickness having been successfully treated by Masters (1918*a*) by the use of this solution alone.

Yorke and Blacklock (1914) tested on small animals samples of trixidin, either forwarded to them by Kolle or prepared by themselves, against several species of trypanosomes, including ***T. evansi***, ***T. equiperdum*** and ***T. congolense***. They obtained best results with suspensions of the drug in oil, although there was often abscess formation, as also relapses, after apparent recovery lasting for 100 days or more. They found that the trypanosomes disappeared from the blood of infected animals within a couple of days of treatment.

Russo (1914*a*) tested both trixidin and metallic antimony of Merck on rats infected with nagana and found that the latter drug had a better sterilizing action but that, when injected subcutaneously, it caused necrosis in 20 per cent of the rats.

Hoffmann (1915) points out that, unlike other trivalent compounds of antimony, trixidin is both non-toxic, as already stated by Hartoch *et al.* (*ante*), and therapeutically active. Like his predecessors he, too, observed that, although the drug was inoculable intramuscularly into small animals, abscess formation took place as a result of the injection. In view of the fact that trixidin precipitated quickly in salt solution and of the consequent danger of the formation of emboli, Hoffmann used a suspension of the salt in sterile gum arabic and sugar ('Speziell Zucker-Gummi') in a strength of 150 mgm. in 1 c.c.

Yakimoff *et al.* (1918) in Russian Turkestan tested trixidin on parasites of camel surra (called by them 'surah'). Atoxyl, arsenosolvin, salvarsan, trypanblue, dianilblue (trypanil) and even tartar emetic were found unsuitable, the therapeutic coefficient of this last being only 1·37. He obtained cure in mice with trixidin in a 30 per cent oily suspension, when used at 0·001 gm. per 20 gm. body weight.

In view of the reputed trypanocidal properties of both antimony and bismuth, Hal (1927) tested what he designated as 'bisuntol'—containing 47·5 per cent bismuth and 13·6 per cent antimony—against *T. congolense* and *T. vivax* infections in cattle and goats, but it was found ineffective.

Two new antimonials, designated Dn 7 and Dn 9, have been fairly extensively tested and found generally effective against trypanosomes (e.g. *T. pecaudi*) in small animals, as also against natural cases of human trypanosomiasis. These compounds are stated to be derivatives of diethylamine oxyquinoline sulphonate containing 16·7 per cent and 34·4 per cent antimony respectively (Van Hoof, 1932). When injected into white rats, the maximum tolerated dose of Dn 7 was found to be 300 and that of Dn 9 261 mgm. per kilo. Both Dn 7 and Dn 9 are claimed by Van Hoof (*loc. cit.*) to have given encouraging results in the treatment of sleeping sickness. Another antimonial in this series, called by Van Hoof (1933*a*) Dn 12, was found by him effective against *T. congolense* but not against *T. brucei* and *T. rhodesiense* in rats. It is also claimed by him to be curative for early cases of human trypanosomiasis. Van Saceghem (1934*a*) tested Dn 7 Dn 18 and Dn 20 in this group of antimonials against bovine trypanosomiasis due to *T. vivax*, but the results do not appear to have been encouraging. Reference may also be made here to Dn 6 and Dn 10, which are mentioned by Dubois (1931) as having been found effective when tested on small animals infected with *T. congolense* and *T. pecaudi*. Van Hoof and Henard (1933) tested Dn 12, Dn 18 and Dn 20 on goats and pigs infected with *T. congolense*—apparently for the first time in cases of natural trypanosomiasis of animals. The results were encouraging, but in no instance a permanent sterilization appears to have been obtained.

According to Rodhain, Dubois and Hendrix (1933), the pentavalent antimonial derivatives of quinoline, Dn 9, Dn 12 and Dn 17, were almost as effective as the trivalent derivatives Dn 7, Dn 8, Dn 11, Dn 14 and Dn 18, while the toxic doses of these two groups were also very near each other. All these antimonials proved to be trypanocidal when tested on rats infected with *T. congolense, T. evansi, T. brucei* and *T. rhodesiense*. Van Hoof (1933*b*) mentions having found Dn 12 effective in sleeping sickness not only by reason of its trypanocidal action but also by reason of the favourable influence it exerted on the cerebrospinal fluid when the drug was injected intravenously twice weekly in 0·5 gm. doses, until 5 to 10 gm. had been given. He (1933*c*) mentions the trivalent antimonial Dn 18 as having proved better than Dn 12 in human trypanosomiasis, for it lent itself to being injected intravenously up to 9 or 10 gm., at 0·5 gm., twice weekly, without toxic effects.

Among other antimonials, mention may be made of stibenyl. Ramsin and Schnitzer (1924) found it less active as a trypanocidal agent than tartar emetic when tested on mice infected with nagana, but no relapses occurred in animals treated with this drug. Van den Branden (1926*b*) found it ineffective in sleeping sickness, although stibosan proved efficacious.

Dunning and Macht (1928) tested certain derivatives of stibanilic acid (azo dyes) on rats infected with dourine; they were not found satisfactory.

Mention may also be made of stibiothiopropanol sulphonate of sodium, which was found by Lepine (1931) to compare favourably with other antimonials when tested on mice and rabbits infected with *T. evansi* and *T. brucei*. It contains 25 per cent antimony.

Bar (1937) mentions the arseno-stibio compound, aminophenyl stibinate of methyl glucamine ('Std. 386B'), as having proved most effective against *T. brucei*, *T. annamense* and *T. congolense* infections in small animals. The drug is stated to contain 18 per cent arsenic and 20 per cent antimony.

The trivalent antimony compound thiomalate of lithium and the glucamine salts of aminophenylstibonic acid containing pentavalent antimony were tested by Launoy (1937*a*) on mice infected with *T. annamense*. The thiomalate was found effective in the early stages of the infection, but only about 50 per cent recoveries were obtained in the later stages. It is noteworthy that the pentavalent compounds were also found by him to be effective. Hornby (1941*a*) used the thiomalate against chronic cases of trypanosomiasis due to *T. congolense*, *T. vivax* and *T. brucei*; its action was found by him to be similar to that of tartar emetic.

The antimonial stibophen was tested by Mackintosh (1945) and Bell (1945*a*) against bovine trypanosomiasis in Uganda, but did not prove effective.

Friedheim and Berman (1946) prepared the quinquevalent antimonial melaminylstibonic acid—a polymerized sodium salt which protected mice for 69 days against *T. equiperdum* when it was injected at 0·05 gm. per kilo. Its therapeutic index is stated by them to be as high as 200. This drug would appear to be the one designated by Friedheim, Vogel and Berman (1948) as MSb. It is stated by them to be more effective than suramin in *T. equiperdum* infection in mice.

Based on the ratio of lethal to curative dose, the therapeutic activity of the following antimonials, when tested on mice infected with *T. equiperdum*, is stated by Chen *et al.* (1945*a*) to be in the order listed: Derivatives of phenyl stibonic acid (stilbamine, stibenyl, neostibosan and neostam), fouadin, antimony sodium thioglycollate, anthiomaline and tartar emetic. The same authors (1945*b*) made the interesting observation that, when cysteine hydrochloride, freshly neutralized with trisodium phosphate, is intramuscularly injected into mice on the second day after inoculation with *T. equiperdum*, it reduced the trypanocidal activity and toxicity of trivalent antimony compounds (tartar emetic, fouadin, etc.), but had no effect on the pentavalent compounds (stibenyl, stilbamine, etc). 'The effect of cysteine hydrochloride upon the trypanocidal activity of these trivalent antimonials was found to be identical with its detoxifying effect'.

Referring to the possibility of accumulation of antimony in blood and organs, Launoy and Fleury (1941), from the results of their trials with p-amino-phenylstibinate methylglucamine on mice infected with *T. annamense*, came to the conclusion that, whatever the dose of the drug, the antimony content in blood became constant, the residual antimony being then excreted slowly. There was accumulation of antimony in the liver, kidneys and muscles, while 82·6 per cent antimony was found in urine and 4·67 per cent in faeces. According to them, the best method of using antimonials is to administer half doses at 24 to 48-hour intervals.

CHAPTER VIII

Antimonials—contd

TARTAR EMETIC

TARTAR emetic is to this day regarded as the cheapest and at the same time a highly effective trypanocide. The quickness of its action is unequalled by any other drug commonly used in the treatment of trypanosomiasis. Van Saceghem and Nicolas (1916) showed that its injection caused the disappearance of *T. congolense* in only 11 minutes from the circulating blood of guinea-pigs infected with these parasites, while Iturbe (1918) found that 4-5 mgm. of tartar emetic per kilo. sterilized in 15 minutes the blood of guinea-pigs infected with derrengadera. Even in the early days of the treatment of trypanosomiasis, the superiority of tartar emetic over certain well-known trypanocides was clearly demonstrated by Tsuzuki (1911) when he published a series of quantitative observations on the relative chemotherapeutic value of 6 standard remedies, the animals experimented upon being mice infected with nagana. The therapeutic dose of each of these drugs per 20 gm. mouse was determined by him to be: arsenious acid, 0·000075—0·0001; atoxyl, 0·001—0·003; arsenophenylglycin, about 0·004; fuchsin, 0·003—0·005; trypanblue, 0·003—0·0075; tartar emetic, 0·0001—0·0004. The last yielded the best therapeutic results, but apparently still better results were obtained with a combination of arsenophenylglycin, trypanblue and tartar emetic. According to him, 'the best results are obtained by the combination of at least 3 remedies' and in such cases 'the doses used are always very small, much less than it must be employed if the same substances are given singly'.

Tartar emetic has 3 important limitations. As shown by Van Saceghem and Nicolas (1916), the serum of animals treated with a lethal dose of the drug has no trypanocidal action *in vitro*, so that it remains only for a short period in the circulating blood in trypanocidal concentration or, in other words, it is excreted quickly. Secondly, as demonstrated by these workers, sterilization following an injection of the drug is only temporary for the parasites return into the circulation shortly afterwards, so that its penetrating action is too small to reach such parasites as are lodged in relatively safe retreats, such as the closed capillaries. Thirdly, it has been repeatedly observed that tartar emetic is drastic in its action, its use being, therefore, contra-indicated in massive infections, in view of the rapid formation of decomposition products from dead trypanosomes which are liable to cause an occlusion of the blood capillaries.

It would now appear to have been well established that intravenous injection is the only really effective method of using tartar emetic. Thus, Sergent *et al.* (1924*b*), in dealing with '*debab*' of camels, employed the drug intramuscularly in oil in one animal and intravenously in an aqueous solution in 7 animals; they found the former method distinctly inferior. Van Saceghem (1921*a*), however, working in Belgian Congo, used tartar emetic intramuscularly, apparently with good results, in the treatment of *T. congolense* infection of cattle. He used a 4 per cent aqueous solution of the drug and to this he added 4 per cent of pure sodium chloride; a small incision was made in the skin before the insertion of the needle, this procedure being stated to minimize the irritant action of the drug which was given in 5 injections at 5-day intervals. He (1922*a*) records having found the drug ineffective when used intravenously or intramuscularly in doses of 0·5-1 gm. in 3 oxen artificially infected with *T. congolense*.

Magrini (1941) has shown that the toxicity of the two antimonials tartar emetic and fouadin is reduced when the injection of these is accompanied by an injection of sodium pyrodisulphonate. The practical possibilities of utilizing this fact under field conditions in the treatment of animal trypanosomiasis with tartar emetic would appear to merit investigation.

An observation of interest recorded by Silberschmidt (1935) was that an inhalation of a 2 to 5 per cent solution of tartar emetic prolonged life to 3 to 4 times normal in rats and mice infected with *T. evansi*, although such inhalation did not produce actual sterilization.

Plimmer (1909) was one of the pioneers in exploring the possibilities of antimony tartrate in the treatment of animal trypanosomiasis. The drug has since been extensively used against surra and nagana. It is proposed to deal, in the first instance, with the findings recorded by different workers on the trypanocidal value of antimony tartrates in combating surra, as being of especial interest for workers in India.

Tartar emetic enjoys to this day considerable popularity as a drug of real value in the treatment of *T. evansi* infection in different species of animals. It is remarkable that, in the early days of the treatment of equine and camel surra in India, the drug was found by Holmes (1910*c*) and Leese (1910*b*) to be ineffective, this being in contrast with the results reported at a later date by Cross (*infra*) as will be seen presently. Holmes mentions the drug as having produced only temporary, though rapid, cure in equine surra when it was used intravenously in a 1 per cent solution, while Leese reports having treated 21 surra-infected camels intravenously with the drug for periods ranging from 2 to 6 months but failed to obtain recovery in any of these.

The results obtained by Cross (1920*a*) in the treatment of camel trypanosomiasis in India by the intravenous injection of a 1 per cent solution of tartar emetic would appear to have been remarkably successful. They are summarized in Table III.

TABLE III

Results of tartar emetic injection in camel trypanosomiasis

Camel No.	Quantity injected and number of injections	Daily or alternate day	Result Post-treatment observation period	Remarks
1	200 c.c. 6	Daily	Cured 129 days	Died after this period without trypanosomes in blood.
2	600 c.c. 7	Do.	Cured 179 days	Do
3	250 c.c. 3	Do.	Cured 780 days	..
4	200—250 c.c. 6	Do.	Cured 513 days	Died after this period without trypanosomes in blood.
5	200—250 c.c. 10	Alternate day	Cured 887 days	..
6	60 c.c	Do.	Cured 945 days	..

Cross recommends injections of large doses, such as 600 c.c., in 3 parts at 2 to 3-hour intervals, for death is likely to occur if the quantity is given in a single injection.

Cross (1920*b*) supplemented the foregoing observations in his annual report for 1919-20. In this report he states that recovery was not obtained with 10 doses of 60 c.c. of the emetic solution or with a single dose of 600 c.c. given in 3 injections at 2 to 3-hour intervals. On the other hand, encouraging results were obtained when 10 to 12 doses of the drug on alternate days were used, commencing with 150 c.c. and increasing to 300 c.c. Working with Patel, however, Cross (1922) found that a suitable commencing dose was 50 c.c., this being progressively raised to 200 c.c.

From the fact that both Cross and Leckie (*infra*) recommend repeated injections of tartar emetic at short intervals, one would conclude *a priori* that the drug remains in trypanocidal concentration in the circulating blood for a short time. As a matter of fact, Edwards (1926) actually found this period to be only 24 hours and he considers the drug to be 'far behind' suramin. Tartar emetic is, therefore, as Edwards (*loc. cit.*) has shown, of very limited prophylactic value. The drug is drastic in its effect and, according to Edwards, it should not be administered in massive infections for the animal may die as a result of blocking of the capillaries by the dead trypanosomes.

Sundaram (1936) used intravenous injections of tartar emetic successfully in dealing with bovine trypanosomiasis in the Saugor district in the Madhya Pradesh. In the same State, Singh (1936) found the drug effective in the early stages of acute bovine surra, but not in chronic cases. Viswanathan (1947) also records having obtained encouraging results with tartar emetic in acute bovine trypasonomiasis but not when the infection was massive.

Tartar emetic has also been a favourite drug for the treatment of surra or closely allied diseases in other countries. Velu (1918) used tartar emetic, either alone or in combination with other drugs, in dealing with a serious outbreak of trypanosomiasis—apparently *T. evansi* infection—in a cavalry in Morocco. The results are summarized in Table IV.

TABLE IV

Results of tartar emetic injection in T. evansi *infection of horses in Morocco*
(Velu, 1918)

Drug	Atoxyl with emetic, massive doses	Emetic massive, with arsenicals small doses	Emetic with massive thiarsol	Emetic with small doses galyl	Emetic with orpiment	Emetic with novarsenobillon	Emetic with arsenicals, both small doses
Number of horses treated	10	11	5	5	5	5	5
Horses that survived after a year	2	4	1	1	0	2	2

N.B.—'Arsenicals' in the table represent different kinds of these drugs in therapeutic use.

The deaths were either attributable to the disease itself or to poisoning by the drugs. From the above table it will be seen that, out of a total of 46 horses treated, only 12 (26·0 per cent) survived—a result which was far from satisfactory.

Iturbe (1918) claims to have obtained very good results with tartar emetic in the treatment of derrengadera* (mal de caderas) in Venezuela. He used the drug intravenously after dissolving 1 to 1·50 gm. of it in 100 c.c. of a 0·4 per cent common salt solution.

Sergent *et al.* (1921*a*) treated 2 experimental cases of camel '*debab*' with a combination of tartar emetic and atoxyl, the former being injected intravenously at a dose of 2 gm. and the latter subcutaneously at 3 gm., at weekly intervals, during a period of 3 weeks; cure was obtained in both. The same workers, in collaboration with Donatien and Lheritier (1921*b*), treated a group of 6 '*debab*' camels with tartar emetic at a dose of 1 gm. at weekly intervals. The interval was apparently determined by the fact that, although the animals were sterilized by an injection of 1-gm. dose, relapses occurred after 8 days. They subsequently had recourse to a series of 4 injections at weekly intervals but in this case, too, the animals relapsed. They, however, adhered to this schedule until the animals received 17 to 39 gm. Four of the 6 animals were cured and these had received from 17 to 28 gm. of the drug in 9 to 10 months. The 2 that failed to recover had received a total of 38 to 39 gm. of tartar emetic. The authors concluded that the treatment was particularly successful with the smallest dose of the drug.

Leckie (1925) lays down the following schedule for the treatment of surra in camel with tartar emetic, this being claimed by him as having given 95 per cent cures: 1st day, 20 cc.; 2nd and 3rd days, 20 c.c.; 5th, 7th and 9th days, 50 c.c.; 11th, 13th and 15th days, 100 c.c.; every 48 hours from 17th to 35th days, 150 c.c.; 37th, 39th and 41st days, 175 c.c.; 43rd, 45th, 47th and 49th days, 200 c.c. The drug is used in a 1 per cent solution, the total amount of drug injected being 30 to 40 gm. Leckie refers to a missionary report from India testifying to the superiority of sodium antimony tartrate over potassium antimony tartrate in the treatment of kala-azar which Leckie designates as a form of 'human trypanosomiasis.' Also, in the treatment of surra he found the sodium salt 'less depressing than the potassium salt.

Delpy (1929) dealt, apparently successfully, with an outbreak of equine surra in Sudan by the injection of a combination of 4-gm. doses of '914' and 1·5 to 2-gm. doses of tartar emetic given on alternate days until 24 gm. of '914' and 628 gm. of emetic had been given.

In the treatment of nagana and other forms of trypanosomiasis, tartar emetic has been by far the commonest drug in use, one important reason of this being its very low cost as remarked by Morris (1936, 1937) in Northern Rhodesia.

Amongst early workers who used tartar emetic, mention may be made of Martin and Leboeuf (1908), who claim having cured *T. congolense* infection in a bull by a single injection of 40 cgm. of the drug. Broden and Rodhain (1909) likewise record a case of recovery in *T. congolense* infection in an ox.

Laveran (1909*b*) reported having obtained good results from the use of this drug in treating *T. congolense* infection in laboratory animals.

Broden and Rodhain (1910), in dealing with *T. congolense* infection in bovines, found that a single 1-gm. dose of tartar emetic, injected subcutaneously, prolonged life of the animal till it was slaughtered for food ; in any case, it kept the blood sterilized for several days. They found the intravenous route unsuitable, for death often resulted in animals injected by this route. Their finding in this respect, however, is contrary to the experience of later workers who have almost unanimously advocated the intravenous route as the best for the purpose.

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*Causal agent *T. Equinum*, bnt Mensil considers it allied to *T evansi* and provisionally designates it as *T.- venezuelense.*

It would appear from a report of the Tsetse Fly Committee of the Economic Advisory Council (1903) that, in Africa, antimony tartrates were first introduced in 1909 in Nigeria. The remarkably large extent to which tartar emetic has since been used in this locality is shown by the fact that, during 1944 alone, as many as 13,326 head of cattle were treated there with this drug (Simmons, 1946) and mention of the use of this drug repeatedly occurs in annual reports of the Nigerian Veterinary Department. Thus, Hall (1933) found both tartar emetic and antimosan equally effective in *T. vivax* infection (apparently in cattle), while Henderson (1933) observed that tartar emetic was both effective and the most inexpensive drug to use in cases of trypanosomiasis due to either *T. congolense* or *T. vivax*. He (1935) states that tartar emetic was till that year the drug of choice, from the point of view of both efficacy and cost, in the treatment of trypanosomiasis in horses and cattle, although repeated injections of the drug were liable to cause a destruction of the liver tissues resulting in mortality. He records having obtained good results in treating 14,000 head of cattle by 3 or 4 intravenous injections of a 4 per cent solution (dose not stated) every 5 or 10 days. Henderson stresses the importance of using freshly made solution of reliable brands of the drug. Tartar emetic stored in open containers was found to have largely lost its trypanocidal value. Marshall *et al.* (1935) used tartar emetic intravenously, in doses of 0·6 to 0·08 c.c. of a 4 per cent solution per kilo., in the treatment of *T. congolense* and *T. vivax* infections in bovines. The drug was administered at 2, 3 or 7-day intervals. The mortality in treated animals was 28 to 37 per cent. The mortality in untreated animals infected with *T. congolense* was 100 per cent and that in untreated animals infected with *T. vivax* about 50 per cent. Mettam, Veterinary Pathologist, Nigeria, in his report for 1938 (issued in 1940), states that, while tartar emetic is effective in *T. vivax* infection in cattle, it is not so in *T. congolense* infection and that animals infected with the latter parasite have to be weeded out.

Tartar emetic has also been used fairly extensively in the Gold Coast Colony. Stewart (1932), working in this Colony, obtained recovery by the use of sodium antimony tartrate in 24 out of 32 horses infected with *T. vivax*. Results obtained in cases of bovine trypanosomiasis were equally successful. The same worker (1935) treated 16 cattle each with 5 intravenous injections of 12 to 15 gm. of sodium antimony tartrate at 5-day intervals. Two of these were infected with *T. congolense* and the others with *T. vivax*. All were cured except 1 which relapsed but was cured after a second course.

It would appear that tartar emetic, while effective in *T. congolense* and *T. vivax* infections in equines, is of little value in *T. brucei* infection, which last is mentioned by Griffiths (1937) as being more amenable to treatment with tryparsamide. A similar view has also been expressed by Stewart (1938).

Turnbull (1933) records having used antimony tartrate, in the Nyasaland Protectorate in doses of 25 c.c. of a 4 per cent solution at 5-day intervals, while Macaulay (1942) working in the Bachuanaland Protectorate, used 20 ml. of a 4 per cent solution of tartar emetic intravenously as a weekly injection in combating *T. congolense* infection in cattle. Turnbull usually gave a course of 6 injections.

Pirani (1934), describing his experience in the treatment of what he designates a *T. abyssinium* infection in cattle, states that, while 5 to 10 c.c. of a 10 per cent solution of atoxyl had no effect on the trypanosomes, the same dose and strength of tartar emetic cleared the circulation. Like most workers, he enjoins caution in the use of the drug on account of its drastic action.

Van Saceghem (1935*a*) mentions a strain of *T. congolense* in cattle which proved resistant to repeated intravenous injections of 1-gm. dose of tartar emetic. He considers it likely that the resistance was due to the animal having been previously treated with the antimonial Dn 7. He (1935*b*) found that, when a large number of cases of *T. congolense* infection in cattle were treated each with 8 intravenous injections of 20 c.c. of a 5 per cent solution of tartar emetic, about 58 per cent recovered after 1 course, 29 per cent after 2 courses and 1 per cent after 3 courses. The mortality during treatment amounted to about 10 per cent, the majority of animals dying after the first injections.

In treating both equine and bovine trypanosomiasis—probably nagana—Bardoz (1934) used tartar emetic in combination with atoxyl, the first every third day and the second every tenth day.

Amongst workers who have made a critical study of the value of tartar emetic as a trypanocide, the names of Hornby, Curson and Bevan deserve special mention. The findings of these workers are summarized below.

Hornby (1919*b*) used tartar emetic continually for 5 years in the treatment of trypanosomiasis—evidently nagana—of domestic animals in Northern Rhodesia, during the First Great War and his experience in this regard is particularly valuable. According to him, a suitable single dose of the drug is 1 gm. for equines and cattle and 0·1 gm. for sheep and goats. The drug is best administered intravenously in a 4 per cent solution. Hornby thus expresses his views concerning the value of tartar emetic as a trypanocide : 'On account of its solubility, low toxicity and high trypanocidal action, tartar emetic is probably the most valuable drug for use on a large scale in the treatment of domestic animals affected with trypanosomiasis. Its cheapness is also a point in its favour'. In the annual report of the Veterinary Department, Tanganyika, for 1931, Hornby and Bailey (1932) recommend 25 c.c. of a 4 per cent solution of tartar emetic—presumably at weekly intervals—to deal with *T. congolense* and *T. vivax* infections in cattle.

According to Hornby (1933), the drug acts directly on the trypanosomes (*cf.* Bevan, *supra*) and, therefore, independent of the antibodies. In view of the possibility of the trypanosomes being lodged in closed capillaries and of the drug being incapable of reaching such locations, the phenomenon of antibody production has also to be largely depended upon for effective sterilization. The formation of the antibodies is substantially lightened by the initial clearance effected by the drug.

Hornby (1935) makes some interesting remarks concerning the possibility of the production of antimony-resistant strains of *T. congolense* and *T. vivax* following the use of tartar emetic. He mentions Yorke *et al.* as having demonstrated that the use of subcurative doses of tartar emetic does not result in the production of strains resistant to this drug, but that an atoxyl-resistant strain readily becomes also resistant to tartaremetic. Hornby, therefore, stresses the desirability of avoiding the use of arsenicals before treatment is undertaken with tartar emetic. He (1945) also makes some suggestive remarks in explanation of the discrepant results obtained from the use of tartar emetic in the treatment of bovine trypanosomiasis. He particularly refers to the significant fact that, while the drug was successfully used in 1913 in the treatment of 50 cattle believed to have been infected with *T. congolense* it proved ineffective in 14 infected cattle in 1941. In the first instance, the animals concerned had passed through a fly belt before they received the emetic injection, so that the drug acted therapeutically. Hornby argues that a proportion of the parasites always escape the action of the drug and that it, therefore, follows that a solid sterilization is only effected by the drug with the co-operation of antibodies. On the other hand, the animals in the second instance had been dosed with tartar emetic before exposure to the flies and they were again injected after removal from the fly belts. Several of the animals relapsed in spite of repeated courses of tartar emetic treatment. This failure, according to him, is attributable to the retardation of antibody production in consequence of early injection of the drug initially. Hornby states that, notwithstanding these facts, the use of tartar emetic has proved to be of inestimable value in combating bovine trypanosomiasis in different localities in Africa.

For the treatment of nagana, Curson (1922, 1924), used tartar emetic intravenously in a 5 per cent normal saline solution as follows: 5 daily injections of 1·5 gm. in cattle and equines and 0·1 to 0·125 gm. per 25 lb. body weight in dogs. In a 94-page inaugural dissertation, Curson (1926) makes an extensive survey of the literature on tartar emetic therapy and makes a series of recommendations, largely based on his own experience. Curson's recommendations on the use of the drug are as follows: (1) a 5 or 10 per cent solution; (2) 1 gm. for a year-old bovine and 1·5 gm. for adults; (3) 1 gm. for donkeys; and (4) 3 to 5 injections, commencing with 1 gm. and progressively increasing to 2 gm. According to him, the toxic action of tartar emetic is attributable to the drug itself and not to toxins resulting from the destruction of trypanosomes. He states that, while in 1920 the mortality amongst infected cattle was 90 per cent, it came down to 20 per cent in 1922 when tartar emetic was issued to stock owners. The value of Curson's method of treatment with tartar emetic was confirmed by Richardson (1928), who obtained recovery in 734 out of 782 cattle treated by this method in South Africa. He used a flat dose of 15 grains of the drug in 25 c.c. solution intravenously.

In a manuscript report submitted in 1918, Bevan states that tartar emetic is particularly adapted for use in bovines, as they are peculiarly tolerant to antimony, although the cure resulting from the use of the drug in these animals is in the nature of tolerance to the presence of the parasites and not one of complete sterilization. Bevan (1928) has thrown some interesting light on the mechanism of action of tartar emetic in trypanosomiasis. The very facts that large doses of the drug, due to its drastic action, are contra-indicated and that only a 1-gm. dose in a 100 per cent solution need be used, indicate that complete sterilization of the circulating blood is not necessary. The drug would appear to operate by killing off a proportion of the trypanosomes and keeping the others in check by liberating toxins and the production of antibodies. That the drug does not effect a change in the trypanosomes is shown by the fact that the disease reproduced in animals by means of trypanosomes derived from a successfully treated animal runs a normal course, so that recovery is attributable to resistance developed by the treated animal. Furthermore, that recovered animal is resistant to re-infection lends support to this conclusion. A method recommended by Bevan for making animals 'tolerant' to infection is as follows: 'The method involves the standardization in sheep of the strain of trypanosomes of the area in which animals are to be exposed to natural infection, and treatment with potassium antimonyl tartrate appropriately timed upon the basis of the appearance and reappearance of the parasite in the peripheral blood'.

The results reported by Fernandes (1942) as having been obtained by him from the use of tartar emetic in the Portuguese colony of Mozambique constitute a striking testimony to the extent to which the drug has proved of value in the running of copra estates in that colony, where cattle had to be maintained for manurial purposes. Tartar emetic was introduced into these copra estates when they were about to close down owing to the difficulty of keeping cattle protected from trypanosomiasis. The introduction of the drug enabled the owners to restock the estates even when tsetse flies were present there. The drug was used intravenously in a 5 per cent solution, at a dose of 8-12 ml. for calves and 30-40 ml. for adults weighing over 500 kilo. Initially, 3 injections at fortnightly intervals were given and 3 to 4 yearly injections for the maintenance of the cattle. When re-infections were common, the injections were given at monthly or fortnightly intervals.

The value of antimonials as trypanocides thus remains vindicated even to this day in spite of the introduction of antrycide which bids fair to supersede most of its other rivals in the field of chemotherapy. Davey (1948), one of the discoverers of antrycide, himself remarks that, while in the treatment of cattle trypanosomiasis antrycide is mostly used against *T. brucei*, antimony tartrate still continues to be used against *T. congolense.*

There are very few records of tartar emetic having been used in the treatment of human trypansomiasis. Van den Branden (1918) injected tartar emetic intravenously to a human patient in a 4 per cent concentration in an oily suspension. The injection was followed by a violent cough and he, therefore, condemned the drug as being contra-indicated for use in trypanosomiasis of man. He found that the oily suspension had no advantage over the aqueous solution.

Laveran (1908) considers the intravenous injection of tartar emetic into human subjects inconvenient in practice and this view would appear to be shared by Thiroux (*Sleeping Sickness Bull.*, 1, 160; 1908).

Occasional instances are on record of recovery having been obtained in human trypanosomiasis by the use of tartar emetic. Thus, Masters (1918*b*) records having used tartar emetic with some success in the treatment of sleeping sickness in Belgian Congo. He treated 370 patients by intravenous injections, on alternate days, of a 2 per cent solution of the drug at a dose of 4 to 12 c.c., the patient being allowed a week's rest after 5 weeks of treatment. The total quantity of drug given to each patient varied with the stage of the disease. Of the 370 patients treated, 160 died or left, while recovery was obtained in 8 of the remaining patients. Although these results can by no means be regarded as encouraging, Masters nevertheless remarks that 'the pendulum has now swung from soamin to antimony tartrate'. Murray (1919) records a case of sleeping sickness in a European which he cured by the intravenous injection of antimony tartrate twice a week, in combination with soamin or antimony oxide, on other days of the week. Bassett-Smith (1922) records a cure of sleeping sickness (*T. gambiense* infection) obtained with tartar emetic, 10 injections of which were given, at a dose of about ½gm. each spring, during 3 consecutive years. The case had proved resistant to atoxyl. Martin and Monier (1931) mention a case of sleeping sickness cured with tartar emetic ; it had proved resistant to arsenicals. Cure of sleeping sickness was attempted by some workers (e.g. Walravens, 1930) by the oral administration of tartar emetic but this proved unsuccessful.

CHAPTER IX

Antimonials—contd.

ANTIMOSAN

ANTIMOSAN (**'Heyden 661'**) is a compound of trivalent antimony with a pyrocatechin derivative. It is more energetic than the 2 well known antimonial preparations, stibenyl (acetyl-p-aminophenylstibiate of potassium or sodium) and stibosan or 'Heyden 471' (m. chloro-p-acetylaminophenylstibiate of sodium), both of which contain antimony in pentavalent form. When administered by the intramuscular or subcutaneous route, it produces little or no local reaction.

Bock (1927) has brought forward experimental evidence to show that antimosan probably undergoes some chemical changes when injected into an animal He found that, in mice and rats, it is more toxic ('unerheblich toxischer') when injected subcutaneously than when it is given intraperitoneally. By the addition of absorption-preventing ('resorptionsverrindernder') colloids, the toxicity of antimosan on intraperitoneal injection can be raised to that of subcutaneous injection. By preventing the otherwise very quick liberation of the drug by ligaturing both ureters, the following toxicity results were produced : (*a*) *On subcutaneous injection.* No increase in toxicity ; perhaps the ligatured animals tolerated even more than normal animals. (*b*) *On intraperitoneal injection.* It produced more than fourfold increase in toxicity. These observations are suggestive of the possibility that antimosan undergoes a chemical change in the organism.

Referring to this drug, Parkin (1930*b*) remarks that the necessity of using the intravenous route for administering a drug greatly detracts from its value and on this point the superiority of antimosan over tartar emetic was at once established when he found that the former lent itself for subcutaneous injection without the occurrence of any untoward local reaction. It is likewise pointed out in a report of the Economic Advisory Council of Tsetse Fly Committee (1933) that the outstanding practical advantage of antimosan over tartar emetic is that the former can be given subcutaneously.

In an introductory note to an article by him on the efficacy of antimosan in bovine trypanosomiasis, Parkin (1930*a*), who worked extensively with this drug in South Africa, gives some useful particulars to justify the replacement of tartar emetic by a more suitable drug, such as antimosan. He quotes Curson as having stated that 'tartar emetic is admittedly far from being an ideal drug chiefly on account of its temporary beneficial action and the disadvantages resulting from faulty administration'. Parkin also refers to the disadvantages of the lengthy course adopted for the use of this drug in certain localities in Africa—varying from 1 injection on each of 5 consecutive days (such as in Zululand) to 8 weekly injections (such as in Tanganyika). He refers to the high cost as the only criticism that can be advanced against antimosan for use in routine practice, this being '10s.per bovine per annum', as against 'the cost of an average dose [of tartar emetic] being approximately one-fifth of a penny'.

Parkin (1935*a*) records having found that the drug, when tested on guinea-pigs infected with *T. congolense,* proved to be free from the drawback of producing drug-fast strains 'either when each generation [of guinea-pigs] was treated once or when the trypanosome was submitted to the largest number of treatments in each guinea-pig'. Du Toit (1936) also, in a note in the report of a Committee on trypanosomiasis, mentions antimosan as a highly satisfactory drug for use in the treatment of *T. congolense* and *T. vivax* infections in cattle and he considers it free from the drawback mentioned above.

Parkin (1930*a*) briefly reviews the experimental observations on record regarding antimosan therapy and the following information is abstracted from his review. Curson (*S. Afr. J. Sci.*, **23**, 603; 1926) found that the drugwas effective against *T. congolense*, but not against *T. brucei*, infections in mice. In a departmental report published in 1927 from Tanganyika, encouraging results are recorded to have been obtained when 9 infected bovines were treated with doses of 50 to 60 c.c. of a 5 per cent solution of antimosan at intervals varying from 2 to 7 days. In the report for the following year, it is stated that the routine procedure in the treatment of bovine trypanosomiasis with antimosan consisted of weekly intravenous injections of 10 doses or less of the drug at 0·015 gm. per kilo.

The results of Parkin's (1930*a*) own trials with antimosan on bovines artificially infected with *T. vivax* and *T. congolense* are summarized below.

I T. VIVAX INFECTION

Antimosan at 8-day intervals

Bovine	Number and weight of doses	Result	Observation period
1	1 of 3 gm.	Died of araplasmosis	
2	3 of 1·8 gm.	Sterilized	76 days.
3	3 of 1·8 gm. and 2 of 1·2 gm.	Do.	84 days
4	1 each of 1·2, 1·8 and 3·0 gm.	Do.	Approximately as above
Control			230 days

II T. CONGOLENSE INFECTION

(*a*) *Antimosan at intervals of 4 or 8 days*

Total Number of bovines	Number and weight of doses	Interval	Result
2	2 and 5 doses respectively of 3 gm.	8 days	Sterilization
2	5 doses of 1·2 gm.	4 and 8 days respectively	Unsuccessful
Control			Parasites present after 5 months

(*b*) *Antimosan in doses of 3 gm. at 7-day intervals*

Sterilization was obtained in 2 bovines during an observation period of 11 weeks. The treatment was unsuccessful in 3 others.

(c) *Antimosan in 1 or 2 doses of 6 gm. each*

Sterlization was obtained in a bovine treated with a single dose and in another with 2 consecutive doses, the observation period being 51 and 71 days respectively. The treatment was unsuccessful in 2 others.

(d) *Antimosan in 2 to 4 doses at 20-day intervals*

Total number of bovines	Dose in gm.	Result	Remarks
9	3	Sterilization	3 relapsed after 1st dose ; 2 after 1st and 2nd dose ; 1 after 1st, 2nd and 3rd dose
4	1·5	Sterilization in 3	Of the 3 cures, 2 relapsed after 1st and 2nd dose ; 1 after 1st and 3rd dose

From the results summarized above, Parkin concludes that a dose of 1·5 gm of antimosan at 28-day intervals is 'decidedly promising for practical application under field conditions'. Parkin's observations also appeared to him to provide indication of the desirability of postponing treatment until the disease had advanced.

Parkin (1930*b*) tested the efficacy of antimosan in 5 horses artificially infected with *T. brucei*. They were treated with 2 to 5 doses of 3 to 6 gm. each, administered at weekly intervals ; 1 was sterilized, 1 died and 3 attained a stage of premunition. There were 3 controls and all of them died. For the treatment of *T. brucei* infection in horses, he recommends an injection of 3 to 6 gm. of antimosan per 300 to 400 kilo. 'at intervals of less than 4 weeks'. He (1931*a*, 1935*b*) followed up these results by carrying out a further series of trials on 5 *T. brucei*-infected horses weighing from 352 to 431 kilo. Sterilization was obtained in 3 and these had received 6 doses of 5·25 gm. each at 21-day intervals, 8 doses of 5·25 gm. at 14-day intervals and 3 doses of 3, 6 and 9 gm. at weekly intervals. The trypanosomes failed to disappear from the blood of the other 2 horses, one of which had received 6 and the other 8 doses of 3·75 gm. at 21-day and 14-day intervals respectively. The three cures, however, showed no resistance to the infection 42-342 days after sterilization.

Parkin (1931*b*) tested the curative effect of a 12 per cent solution of antimosan for sheep artificially infected with *T. congolense*. He failed to obtain sterilization in a sheep treated with a single dose of 3 c.c. of the drug, while one recovered out of 2 others that had received single doses of 7 c.c. of antimosan. Sterilization was obtained in 13 out of a group of 17 sheep treated with a 10 c.c. dose. Fifty-six sheep received 2 or more of 3, 5, 7 and 10-c.c. doses of the drug at intervals of 7 or 28 days, and the results are summarized in Table V.

TABLE V.

Summary of treatment of 56 sheep artificially infected with T. congolense

Doses in c.c.	Number of sheep	Number sterilized
3, 5, 5, 5 ..	2	0
3, 5, 5, 5, 5	7	3
5, 5.5, 5 ..	3	0
5, 5, 5, 5, 5	9	2
3, 5, 10, 10	2	2
5, 5, 10, 10	3	3
7, 7 ..	12	9
10, 10 ..	2	1
5, 10 ..	10	9
10, 5 ..	6	3

The first 6 groups of sheep were treated at 7-day and the other 4 groups at 28-day intervals. Parkin concludes from these observations 'that the practical and economical dosage for the production of sterilization is the 10 c.c. repeated'.

Finally, Parkin (1935*c*) used antimosan in the treatment of both natural and artificial T. *congolense* infections in dogs and found the results 'quite remarkable', for not only it sterilized the circulation, but also caused the 'entire disappearance' of the symptoms. He gives the protocols of 2 dogs, both of which were repeatedly artificially infected and then treated. The dosage of the drug was 0·025 gm. per kilo. in all the treatments in one and 0·17, 0·29 and 0·046 gm. per kilo. in the other.

Bevan (1931) in Southern Rhodesia found that antimosan was only effective in the treatment of bovine trypanosomiasis in the early stages of the disease and was, therefore, of little value under field conditions, in view of the practical difficulty of diagnosing infection at the correct time when a large number of animals were concerned.

Van den Branden (1926*c*) tested the efficacy of antimosan on 8 cases of human trypanosomiasis and found its action less energetic than that of tartar emetic and also transient ('passagére'). According to him, it can be regarded as no more capable than stibenyl or stibosan of superseding tartar emetic and it even rapidly aggravates the general condition of the patient when used in the treatment of chronic trypanosomiasis. In the treatment of animal trypanosomiasis, however, Daubney (1930), working in Kenya, found antimosan as good as tartar emetic.

In his report on the work of the Veterinary Laboratory, Mpwapwa (Tanganyika Territory), Hornby (1931) recommends for the treatment of bovine trypanosomiasis a course of 5 subcutaneous doses of a 6·3 per cent solution (as standardized by the manufacturers) of antimosan at weekly intervals, the course being repeated after a first and a second relapse, with a single dose of 80 c.c. in the event of a third relapse.

Mettam (1934) found that, under field conditions in Uganda, antimosan yielded highly satisfactory results in the treatment of *T. congolense* infection of cattle, when it was injected in doses of 40 c.c. on 4 consecutive days, although a relapse might be expected to occur in 50 per cent of the animals, and these should be given a second course of treatment. He remarks that, on account of its high cost, it is unsuitable for use in large outbreaks, which have, therefore, to be controlled by means of tartar emetic. Referring to the prophylactic value of antimosan, Poulton (1934), working in the Uganda Protectorate, states that the drug proved incapable of preventing infection in working oxen when it was given in weekly injections followed by injections at fortnightly intervals, but the treated animals remained in good condition. De Meza (1939) records a similar experience in the Nyasaland Protectorate, for he found that prophylactically treated cattle did not lose in health, although they continued to harbour the trypanosomes. He mentions 3 animals having survived for 3 years by receiving 1 dose of antimosan yearly.

Stewart (1935) in Gold Coast treated 29 head of cattle with 5 weekly injections of antimosan 'in 40 c.c. doses to adults down to 5 grains in calves'; 1 relapsed and died and another died from other causes, while all of the remaining 27 recovered.

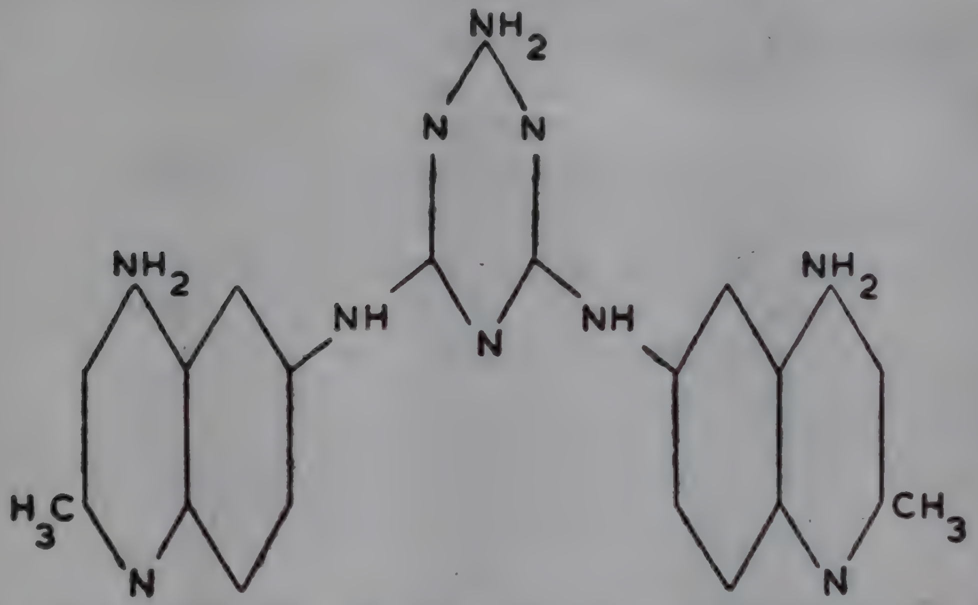
Peck (1943) used the drug in the treatment of *T. evansi* infection in camels. Single doses of 40 to 60 ml. of antimosan were administered subcutaneously to 6 animals, but none appears to have been cured. The drug has otherwise been seldom used in the treatment of this infection.

Le Roux (1943) in Northern Rhodesia records having cured *T. simiae* infection in a pig weighing 150 lb. by the intravenous injection of 2 doses of antimosan at 40 ml. each.

CHAPTER X

Surfen C

SURFEN C ('Hochst 6678'), a quinoline derivative, is a Bayer preparation and is bis-2-methyl-aminoquinolyl-6-melamine with the following formula:—



Surfen C should not be confused with the antiseptic substance Surfen, which is bis-2 methyl-4-amino-quinolyl-6-carbamide. The properties of Surfen C are discussed by Iensch (1937), who states that it is contra-indicated in man because it produces nephritis. Surfen C is identical with the Bayer preparation 'congasin'.

Brauss (1944) tested on mice the trypanocidal action of both Surfen and Surfen C. The former is stated by him to be effective against *T.equiperdum* but not against *T. congolense* which was, however, found to be susceptible to Surfen C. Tested on mice infected with *T. brucei*, Surfen C showed a therapeutic index of 1:50. In doses of 0·05-0·5 mgm. it prolonged life, while 1-10 mgm. had a curative effect. It conferred complete protection on mice when it was injected in a dose of 1 mgm. 48 hours before infection. There was evidence of drug-fastness when it was injected in small doses.

Surfen C was introduced into the field of chemotherapy with the claim that the curative dose of the drug in *T.congolense* infection was only a single injection of 10 mgm. per kilo. as against 4 subcutaneous injections, at weekly intervals, of 3 gm. antimosan, which, prior to the introduction of Surfen C, was a frequently used drug in the treatment of the disease. The claim was also put forward that, in calves, an intramuscular injection of 5 mgm. of the drug per kilo. cured *T. congolense* infections in the early stages. The drug at a dose of 10 mgm. per kilo. was also stated to be equally effective in both early and advanced *T. congolense* infections in sheep.

Hornby (1934) in Tanganyika was one of the earliest to work with Surfen C and he states: 'The result which may be expected to follow a single intramuscular injection of Surfen C is at least as good as the result to be expected from a course of 5 weekly injections of antimosan or tartar emetic'. According to Hornby's (1938*a*) experience, the effect of intravenous injection of the drug is too severe for the animal and he considers that, therapeutically, the intramuscular route is as good as the intravenous.

According to Delidimitriou (1938), the subcutaneous route is as good as the intramuscular for the injection of Surfen C. He found that, when injected intramuscularly in a dose of 100 c.c. of a 2·5 per cent solution to 270 cattle infected with *T. congolense*, the trypanosomes persisted only in 28 of 253 animals examined 1 to 6 months after the treatment. Van Saceghem (1938*a*) also records a similar experience and in addition he found the drug effective against *T. vivax*. Langer (1939) likewise obtained good results with Surfen C in the treatment of *T. congolense* and *T. vivax* infections in cattle and horses.

Bennett (1935) in Sudan records having used Surfen C successfully in treating a natural case of *T. congolense* infection in a horse. He found the drug unsuitable for injection by the subcutaneous or intravenous route. :—

Stewart (1935) in Gold Coast tested the efficacy of a single intramuscular injection of Surfen C in bullocks and the results are shown below.

(*a*) *In 1933*. Seventeen West African shorthorn infected with *T. vivax*. He reports the results thus: 'Five of these bullocks have been positive since, one of them on 2 occasions but these five have been negative since end of 1934'.

(*b*) *In 1934*. (*i*) Thirteen West African shorthorn of which one each was infected with *T. theileri*, *T. brucei* and *T. congolense* and the others with *T. vivax*. (*ii*) 7 Zebu of which 2 were infected with *T. congolense* and others with *T. vivax*; relapses occurred in 4 of (*i*) and 5 of (*ii*). According to Stewart, Surfen C is less effective than tartar emetic, but it 'appears to be useful after antimony treatment to prevent relapses'. The drug was used in doses of 100 c.c. In his annual report for the year 1935-36, Stewart observes that the drug is more effective when injected intravenously, although alarming symptoms are produced when this route is used.

While antimosan has been an extensively used drug in Nigeria (*ante*), Surfen C received particular attention in the treatment of animal trypanosomiasis in the Uganda Protectorate. Mettam (1935 *a*,*b*), working in this locality, reports having experimentally used the drug with success Ninenteen cattle artificially infected with *T. congolense* were each treated intramuscularly with the scheduled dose of 100 c.c. of Surfen C; 1 died and the others were cured. Four of the latter were subjected to test inoculation with the parasites 3 months later and they proved immune. In a later report, Mettam (1936) writes that Surfen C is the best trypanocidal agent available for the treatment of cattle trypanosomiasis. He further remarks that, while antimonials are most efficacious against *T. congolense*, they have but little action on *T. brucei*, but that Surfen C is efficacious against all the 3 cattle trypanosomes, namely, *T. congolense*, *T. vivax* and *T. brucei*. Poulton (1935) mentions having obtained encouraging results with Surfen C in the treatment of cattle infected with *T. congolense* in the Protectorate, where as many as 91 outbreaks due to *T. vivax*, *T. congolense* and *T.brucei* occurred in 1944 alone. In a subsequent report, Poulton (1937) gives an account of tests carried out by him with Surfen C on 2 small herds of cattle showing mixed infections. The drug cleared the circulation of both *T. congolense* and *T. vivax*, but *T. brucei* persisted in a few of the animals. On the other hand, Marshall (1937), working in the same locality, found the drug ineffective against a Nigerian strain of *T. vivax* in cattle. Carmichael (1938) found Surfen C valueless in calves infected with *T. congolense*. On the whole, observations of Uganda workers can hardly be reconciled with those recorded by certain workers elsewhere such as Delidimitriou (1938) and Van Saceghem (1938), to whose names reference has been made earlier. It is, however, possible that, as considered likely by Van Saceghem (1939), the discrepancies may in some measure be due to different samples of Surfen C varying in potency.

Bennett (1936) in Sudan, in comparing the value of Surfen C with that of antimosan in the treatment of bovine trypanosomiasis due to *T. congolense*, states that a single dose of Surfen C at 1 gm. per kilo. is as effective as 20 c.c. per kilo. of a 40 per cent solution antimosan.

Bennett (1936*b*) also tested the efficacy of Surfen C for natural *T. congolense* infection in 14 horses, each weighing approximately 250 kilo. On the recommendation of the manufacturers (Messrs. Bayer), he used a 2·5 per cent solution of the drug. He considers as 'almost certainly unsafe' to administer the drug intravenously. As for the subcutaneous route, 'large swellings developing at the site of injection render this route objectionable'. Even when the intramuscular route was used, he found that "troublesome local swellings may develop". Nevertheless this last route was considered to be the safest and in 13 out of the 14 horses Bennett administered the drug in 4 intramuscular injections, namely, into both thighs and buttocks. In the remaining horse, the drug was given intravenously, with temporary symptoms of collapse and later it received a second dose subcutaneously. Of the 13 horses mentioned above, 9 recovered after receving a single injection of the drug in doses of 2·5 mg. (3 animals), 3·75 gm. (5 animals) and 5 gm. (1 animal). The remaining 4 horses relapsed one or more times and were re-treated with the drug, as shown in Table VI :—

TABLE VI

Results of Surfen C treatment in horses

Horse	Date of 1st treatment and dose of drug in gm.	Date of 1st relapse and dose of drug in gm.	Date of 2nd relapse and dose of drug in gm.	Date of 3rd relapse and dose of drug in gm.	Remarks
1	12-V-35 2·5	14-VI-35 3·75			Cured
2	2-VI-35 2·5	15-VII-35 3·75	28-VIII-35 5·0		Cured
3	13-VII-35 3·75	9-IX-35 5·0	9-IX-35 7·5	7-I-36 to 8-II-36 12·5 (in 5 weekly injections)	Cured
4	13-VII-35 5·0	20-I-36 to 8-II-36 7·5 (in 3 injections at 10 day interval).			Cured

It is of interest that in none of the relapse cases the parasites were found in the circulating blood, but that their presence was detected on sub-inoculation into gerbils.

Evans (1936) in Sudan has published a small number of observations on the relative efficacy of antimosan and Surfen C for *T. congolense* infection in cattle. These observations are summarized below.

Antimosan. Twenty naturally infected bulls, weighing between 700 and 800 lb., received a course of 5 weekly injections and 7 others with the same body weight received 3 weekly injections of antimosan. The dose in each case was 40 c.c. and it was administered by the intravenous route. Cure was obtained only in those that received 5 injections. A course of 5 weekly injections also resulted in the recovery of 12 other bulls naturally infected with *T. congolense*, but in these cases a dose of 10 c.c. per 100 lb. body weight was used.

Surfen C. The drug was supplied by the manufacturers in a 2·5 per cent solution, the recommended dose for a bull being 100 c.c. Cure was obtained in all of a batch of 7 bulls treated with the drug, although 1 died 13 days after treatment, apparently from causes other than trypanosomiasis.

Lester (1935) found a dose of 10 mgm. per kilo. of Surfen C more effective than a standard dose of tartar emetic for sheep infected with *T. congolense* and *T. vivax*, but relapse occurred in these animals after 3 doses of the drug given at weekly intervals. Relapses also occurred in a few weeks of treatment in the majority of a batch of 18 bovines, 6 of which had received the drug at 10 mgm. per kilo. in 3 weekly doses, while the other 12 received single doses of the drug. It is worthy of note that, while a course of 3 injections failed to sterilize, all the treated animals remained alive after 6 months, the percentage of survivals among the controls was only 6·2.

Van Rensburg (1938) refers to the Veterinary Pathologist, Nigeria—presumably Marshall to whose name a reference has been made earlier—as having stated in his 1935 report that, out of a batch of 6 animals artificially infected with *T. vivax* and treated with Surfen C, 2 died of trypanosomiasis and a third continued to show the parasites in blood. Van Rensburg (1938) also refers to Danks and Daubney in Kenya as having stated, in a memorandum submitted by them to the Conference for Co-ordination of Tsetse and Trypanosome Research in East Africa, that as many as 6 relapsed out of 13 bovines treated with Surfen C for trypanosomiasis and that 'Daubney is doubtful about the effect of the drug'. Van Rensburg quotes the following remarks made by Bevan (1937) in his Progress Report on Research on Trypanosomiasis in Southern Rhodesia: 'The so-called Surfen C, much vaunted as a remedy for *T. congolense* infection elsewhere has not proved as successful against the local strains of trypanosome'.

Van Rensburg himself (1938) makes the following statement: '(1) The intramuscular injection of Surfen C in the treatment of *T. congolense* infection in bovines and ovines was found to be ineffective. (2) The intramuscular injection if Surfen C into bovines caused serious local reaction". He tested the efficacy of the drug on 5 bovines and 6 sheep artificially infected with *T. congolense*. The results, which are given by him in 2 tables, are summarized below (the dosages are per kilo. body weight):

Bovine 1. Received a dose of 10 mgm. followed by 5 doses of 2·5 mgm.; then by 2 of 5 mgm. and finally by 2 of 2·5 mgm. combined with 15 c.c. of a 12 per cent solution of antimosan; the intervals between these doses were 49, 1, 19, 1, 1, 19, 1 ,88 and 22 days respectively. The treatment was unsuccessful; the trypanosomes temporarily disappeared for a few days during treatment.

Bovine 2. Received 2 doses of 2·5 mgm. each, followed by 5 doses of 5 mgm. each; intervals 1, 142, 7, 7, 7 and 8 days respectively. Unsuccessful; trypanosomes temporarily disappeared as in Bovine 1.

Bovine 3. Received 5 doses of 5 mgm. each ; intervals 7, 7, 7, and 8 days respectively. Unsuccessful; trypanosomes failed to disappear.

Bovine 4. Received 3 doses of 10 mgm. each at intervals of 20 and 5 days respectively. Seven days after 2nd dose, trypanosomes were absent for 16 days and disappeared altogether after the 3rd dose.

Sheep 1. Received 2 doses of 2 mgm. each, followed by 2 doses of 2 mgm. each; intervals 1, 22, and 1 day respectively. Unsuccessful; trypanosomes temporarily disappeared for a few days during treatment.

Sheep 2. Received 2 doses of 5 mgm. each at an interval of 1 day. Sterilized.

Sheep 3. Received 2 doses of 2·5 mgm. each, followed by 6 doses of 5 mgm. each ; intervals 1, 11, 1, 88, 7, 7 and 7 days respectively. Unsuccessful ; trypanosomes temporarily disappeared for a few days only during treatment.

Sheep 4. Received a dose of 2·5 mgm. combined with 5 c.c. antimosan, this being followed, after an interval of 8 days, by a dose of 10 mgm. antimosan. Trypanosomes temporarily disappeared for a few days after the 1st dose and altogether disappeared after the 2nd.

Sheep 5. Received 2 doses of 10 mgm. each ; interval 50 days. Unsuccessful; trypanosomes disappeared for a few days only during treatment.

Sheep 6. Received a single dose of 10 mgm. Unsuccessful ; trypanosomes absent from circulation for 3 days only during treatment.

Perhaps the most unfavourable report yet published on the effect of a trypanocide is that of Le Roux (1937) on Surfen C. Working in Northern Rhodesia, Le Roux records disastrous results from the use of this drug in cattle trypanosomiasis, some of the animals having died, 15 minutes after drug injection, with froth and blood from nostrils. On post-mortem examination, they showed haemorrhages in the lungs. Guyaux (1940) used Surfen C in the usual dose of 100 c.c. of a 2·5 per cent solution in the treatment of 28 cattle infected with *T. congolense.* He obtained cure in 16 of these, while the remaining 12 showed the trypanosomes for 3 to 8 months after treatment. He cites experimental observations to establish its superiority over tartar emetic.

Cilli (1941), working in Eritrea, found the drug ineffective against *T. vivax* infection in cattle. He further noted that, while the animals tolerated an intramuscular dose of 40 ml. of a 2·5 per cent solution of the drug, it might cause death in those weakened by the disease.

Kraneveld and Mansjoer (1940) found the drug of little prophylactic value and almost valueless therapeutically against surra in guinea-pigs. According to them it is much inferior to suramin.

To sum up, the reports that have emanated from different parts of Africa concerning the efficacy of Surfen C for animal trypanosomiasis are remarkably conflicting. Workers who report more or less favourably on the drug include Hornby and Bailey (Tanganyika), Mettam and Carmichael (Uganda), Kenya Veterinary Service, Evans (Sudan), Bennett (Sudan), Lester (Nigeria), Van Saceghem (Belgian Congo) and Delidimitriou (Belgian Congo). Workers who found the drug more or less ineffective include Marshall (Nigeria), Stewart (Gold Coast), Danks and Daubney (Kenya), Bevan (Southern Rhodesia), Le Roux (Northern Rhodesia) and Van Rensburg (South Africa).

CHAPTER XI

Bismuth Compounds

AS COMPARED with the other two groups of metallic compounds, namely, arsenicals and antimonials, bismuth compounds occupy a minor position in the chemmotherapy of trypanosomiasis, whether of man or of animals, and, judging from available indications, it is more than improbable that bismuth will ever figure largely in any future work on this disease.

Adler (1921*b*) carried out some experiments on the efficacy of bismuth sodium tartrate for *T. brucei* and *T. rhodesiense* infections in rats but found it valueless.

The socalled 'trepol'—tartro-bismuthate of potassium and sodium in oil—was used by Van den Braden and Van Hoof (1922*b*) in 3 cases of sleeping sickness in 3 to 5 doses of 0·3 gm., but it proved ineffective. Van den Branden (1922) evidently refers to these same observations in a paper published by him in the same year. Vladesco and Friminoiu (1923) tested 'trepol' on guinea-pigs infected with nagana or surra. When injected intraperitoneally at 1 to 1·5 c.c., it caused peritonitis, although sterilization was effected. Relapses took place when it was given by the intramuscular or subcutaneous route. It was likewise found valueless by Van Saceghem (1923) when tested on cattle experimentally infected with *T. congolense*. Sazerac and Levaditi (1921*a*, *b*) also used it unsuccessfully, at a dose of 100 mgm. per kilo., against nagana in guinea-pigs.

Van Saceghem (1924*a*) records having failed to use with success the socalled 'bismoxyl'—a combination of 1 per cent solution of bismuth tartrate with extract of ox liver—in *T. congolense* infection. He (1925*b*) also tested the efficacy of colloidal bismuth—called by him 'bismuthoidal'—when injected intravenously in 20-50 c.c. doses into bovines infected with *T. vivax*, *T. congolense*, and *T. brucei*, the bismuth having been previously made isotonic in solution with sugar. It proved effective only against *T. brucei.*

Hall (1927) carried out trials with what he designated as 'bisantol' in the treatment of *T. vivax* and *T. congolense* infections in cattle, but it proved to be of little value (*vide* section dealing with antimonials).

It is stated by Frouin and Guillaumie (1921) that a 1 per cent solution of subnitrate of bismuth in a 10 per cent mannite, when injected into mice infected with nagana, sterilized the circulating blood and also prolonged the life of the animals. This treatment would seem to merit further investigation.

CHAPTER XII

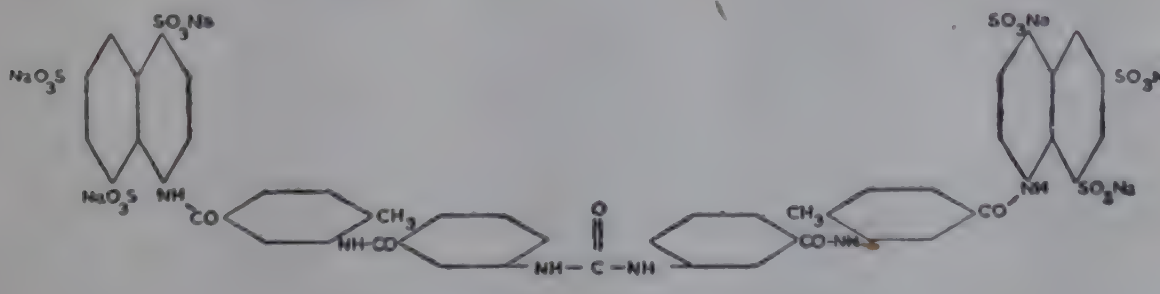
Suramin

THE discovery of suramin—also commonly known in veterinary practice by the names of 'Bayer 205', naganol and antrypol—constitutes an event of the greatest importance in the history of chemotherapy of trypanosomiasis, whether of man or of animals. It superseded almost all the drugs previously in use in the treatment of this disease, the absence of arsenic in it being considered one of its special merits, for on this account its prolonged use was free from the attendant danger of causing visual distrubances or of producing resistant strains to the same extent as arsenicals. Suramin was found safe to use and slow but steady and not drastic as tartar emetic in its effect. It was slowly excreted, so that it remained for a prolonged period in trypanocidal concentration in the circulating blood. Its practical advantages consisted in the readiness with which it dissolved in water and in the fact that it lent itself for injection by the subcutaneous, intramuscular, intravenous or intraperitoneal route.

It will be convenient for the purpose of this section to consider the records of work with suramin under three separate heads: (1) laboratory trials, (2) its use in animal trypanosomiasis and (3) its use in sleeping sickness. This last will be dealt with as briefly as possible, the object being merely to provide some indication of the extent to which the drug has proved of value in the treatment of human subjects.

Suramin was manufactured about 1918 by the firm of Bayer & Co., Elberfeld, Germany and for some years its chemical composition was not disclosed, although it was known that the drug was not an arsenical. Hendel and Joetten (1920) found the drug highly effective when tested on mice, rats and guinea-pigs infected with nagana or dourine and also when tested on a donkey and dogs. Mayer and Zeiss (1920) likewise found the drug possessing unique trypanocidal properties and evidently considered it to be an arsenical preparation although in reality it was a non-metallic compound. Laboratory trials carried out by them on rats and mice showed that it was highly effective against *T. brucei*, *T. equiperdum*, *T. equinum*, *T. gambiense* and *T. rhodesiense*, but ineffective against *T. cruzi*. They further observed that in blood smears the drug affected the multiplication processes, preventing the parasites from undergoing division.

Fourneau, Trefouel and Valleé (1924) in France, after a series of laborious investigations, produced a drug which they designated 'Fourneau 309' and claimed as the equivalent of 'Bayer 205'. Their finding later proved to be correct, for it is now known that both ' Bayer 205 ' and ' Fourneau 309 ' are symmetrical urea of the sodium salt of *m*-benzoyl-*m*-amino-*p*-methylbenzoyl-1-aminonaphthalene-4 : 6 : 8-trisulphonic acid. Its chemical constitution is as follows:



For convenience, the drug will be mostly referred to as suramin throughout this section.

The claim that 'Fourneau 309' was identical with 'Bayer 205' was therapeutically tested by Laigret and Blanchard (1925). They found that the curative action of the two drugs was exactly similar in guinea-pigs infected with *T. pecaudi* and *T. gmbiense.*

1. Laboratory Trials with Suramin

The introduction of suramin into the field of chemotherapy was immediately followed by laboratory trials with this drug by a number of different workers and, for convenience of treatment, the results are summarized here, as far as possible, both subject-wise and in chronological order.

Wenyon (1921) reported having used the drug successfully in the treatment of 50 mice infected with *T. equiperdum.* A single dose of 2·5 mgm. per kilo. was used in each case.

Mayer (1922*a*) tested the efficacy of suramin when administered orally to 2 goats infected with *T. rhodesiense.* Cure was obtained in one that received 20 gm. in 6 days, while a relapse after a month occurred in the other that had received 2 gm. Brumpt and Lavier (1922), working with *T. rhodesiense* and *T. venezuelense,* found that 0·5 mgm. of the drug effected a cure even when injected only a few hours before the expected time of death in mice. They found suramin also curative for *T. brucei* infection in mice and rabbits.

Concerning the therapeutic value of excreted material from subjects treated with suramin, Thomson and Robertson (1922) found that the urine of a patient treated with this drug had no effect on rats infected with *T. rhodesiense.*

In studying the action of suramin on fatal infections in the frog *Rana esculenta* caused by *T. inopinatum,* Brumpt (1923) found that in citrated blood it was slow but efficient and it seemed progressively to destroy the virulence of the parasite (' il semble avoir détruit la virulence ').

Shigemoto (1923) removed organs of drug-injected guinea-pigs and inoculated their extracts into trypanosome-infected animals. From a study of the course of the disease, they concluded that the organ which harboured the largest amount of the drug was the kidney, the next in order being the liver.

In rats infected with *T. rhodesiense,* Corson (1934*a*) found that cure was obtained with 1 c.c. of a 0·2 per cent solution of suramin, but that relapse occurred after 5 days.

Lange and Kersten (1924) refer to the discrepancies in the results reported by different workers on the toxic dose of suramin for mice. Thus, Hendel and Joetten give the toxic dose as 18-20 mgm., although 30 mgm. is sometimes tolerated; Mayer and Zeiss, 10 mgm.; Miessner and Berger, between 10 and 15 mgm. Lange and Kersten themselves regard the toxic dose of suramin as 18-20 mgm. They found that the dose of 1 mgm. protected mice—apparently against the common trypanosomes–for 47 days. Lange and Kersten do not subscribe to the view advanced by certain workers that naturally infected animals, which are more or less in a chronic state of disease, constitute the optimum medium for the growth of trypanosomes which, in consequence, are more resistant to the action of the drug. According to Lange and Kersten, on the other hand, it is the animal in which the parasites are capable of undergoing a rapid and boundless development that must be regarded as most suitable for them. This view, however, would seem hardly tenable if pathogenicity is to be regarded as a sign of imperfect adaptation, for a fulminating type of development on the part of the parasites can only end fatally for the host and, therefore, for the parasites themselves, so that a biological equilibrium between the host and the parasite must be regarded as the optimum condition for the growth and survival of the parasites.

Kligler and Weitzman (1924) found that the drug caused a degeneration of the epithelium of the kidney and they claim to have produced relapse in treated laboratory animals by the administration of cod liver oil.

According to Nauck (1925), suramin not only exercises a beneficial effect on the body, but also causes a progressive decrease in virulence of the parasites and prevents their multiplication.

Pfannenstiel and Scharlau (1929) record the interesting fact that the action of suramin improved when it was combined with *dry* human serum. When, however, 0·3 c.c. human serum in the wet form containing about 25 mgm. drug substance was used, relapse resulted, whereas an injection of serum powder prepared from the same amount of serum produced cure. These authors apparently experimented on mice infected with dourine.

Valenza (1935) found that a *congolense*-infected guinea-pig, after being cured by suramin, was capable of resisting re-infection once, but succumbed to a second re-infection.

Results of what might be described as ' quantitative experiments ' are also on record concerning the minimum trypanocidal concentration of suramin necessary to effect a cure. Moschkaowski (1927) determined this concentration for guinea-pigs and mice infected with *T. brucei* and *T. evansi*. It was found to be almost the same as *in vitro*, nemely, 1/3,000 of the body weight of the animal when the drug was injected subcutaneously. Vierthaler and Boselli (1939) carried out what would appear to be a laborious series of experiments with graded doses of suramin to determine the minimum concentration of the drug in blood necessary to effect a cure in rabbits infected with *T. brucei*. This concentration was found to be 1·3 mgm. per 100 c.c. of blood. They have given figures to show the amounts of suramin present in the blood of rabbits for from 5 minutes to 10 days when the drug was administered in varying doses. They found that rabbits resisted *T. brucei* infection even when the amount of drug present in blood was only 1 mgm. per 100 c.c. Culbertson (1939) studied the effect of suramin on *T. equiperdum* infection in rats of different age groups and made the notable observation that the rapidity of sterilization varied inversely with the age of the animal. In old rats the drug sterilized the circulation in 15 hours, but in young rats it took 30 to 60 hours to do so.

Reference has been made earlier to suramin having been given by the mouth. It is of interest that Trillat (1936) tested the possibility of effecting a cure in infected animals by causing them to inhale the drug. He observed that 3 hours' inhalation of about 0·75 mgm. suramin, dispersed in a cloud, delayed infection with *T. brucei* in 252 mice. Van den Branden (1938*b*) likewise found that suramin vapour, although it did not cure rats infected with *T. brucei*, prevented them from developing the infection.

Launoy (1934*a*) tested the value of suramin for cats infected with *T. evansi*. He observed that a dose of 20 to 30 mgm. of the drug per kilo. sometimes cured these animals of the infection. Launoy (1937*b*) further observed that in cats infected with *T. annamense* injection of repeated doses of suramin produced what they called para-immunity, the animals being thereby made resistant to massive inoculation of trypanosomes.

Trials on the prophylactic value of suramin for laboratory animals infected with different species of trypanosomes have been carried out by several workers. Their findings are summarized below.

Protection against trypanosome infection was obtained by Mayer and Menk (1922 *a*) in a goat that had been dosed with 20 gm. suramin and infected a week later, and also in a dog that had received 1 gm. on each of 3 consecutive days and infected 14 days later. Serum of infected goats treated orally with suramin was found to have therapeutic effect in mice infected with *T. equinum*, *T. brucei* and *T. equiperdum*, while milk of goats similarly treated had a prophylactic effect against. *Tequinum* infection in mice. Brumpt and Lavier (1923) tested the prophylactic effects of the drug in laboratory animals infected with *T. equinum* and *T. pecaudi*. In the first case, it protected for 78 days; in the second, up to 71 days. Kasai and Akazawa (1925) found that 0·005 gm. of suramin protected for 5 months mice infected with what was apparently surra parasites from Formosan water buffaloes. In their studies upon the action of suramin on guinea-pigs against *T. equinum* infection, Dios and Zuccarini (1926) found that 200 mgm. of the drug protected them for about 100 days. Levaditi and Klarenbeck (1927) found that a dose of 0·1 gm. suramin, when administered by the mouth, protected rabbits from nagana for 39 to 87 days, while Akazawa (1928) states that a dose of 0·005 gm. protected mice for 4 months.

Launoy *et al.* (1929) have brought forward some evidence to show that, contrary to the general view, the prophylactic value of suramin is not merely attributable to the presence of the drug in the circulation or in the tissue, but also to other factors which are not understood. This is suggested by the fact that, when a subminimal preventive dose of suramin is given to mice, guinea-pigs or cats before they are infected with *T. brucei*, *T. evansi* or *T. equiperdum*, a refractory period of short duration in these animals is produced, but nevertheless, they may resist the infection for a long time and even recover spontaneously. These authors also observed that, when minimal or subminimal doses of suramin are used, the duration of the refractory period is proportional to the size of the dose. Launoy *et al.* (1930) state that the duration of the refractory period is not arithmetically proportional to the dose of the drug.

Corson (1934*b*) would appear to be one of the earliest to study the prophylactic value of suramin for small laboratory animals against the bites of infected tsetse flies. Working with white rats he found that 0·03 gm. of the drug protected them for 40 days against *T. rhodesiense* conveyed by *Glossina morsitans*.

Launoy *et al.* (1930) have given some quantitative information on the protective value of suramin. They found that, when 4 doses ranging from 0·0001 gm. to 0·0006 gm. were used, the protective value increased in direct proportion to the size of the dose, each increase of 0·0001 gm. increasing the protective period for about 5 days. Their observations were made on mice infected with *T. brucei*. Launoy (1934*b*) stresses the inadvisability of regarding the results obtained from experiments on *brucei*-infected mice as providing an indication of the protective dose of suramin required to be used against trypanosomiasis in large animals, such as *T. equiperdum* infection in the donkey. According to him, the appropriate dose for such animals is to be determined largely 'by surfaces'; otherwise, it should be ascertained by actual experiments.

Trillat (1936) was able to demonstrate the prophylactic action of suramin against *T. brucei* on inhalation for a short time by mice confined in a bell jar, the drug being used in a 10 per cent solution, at 0·75 mgm. per 25 gm. body wieght. Under such conditions, the infection was not only arrested but none of the mice relapsed for 1 month.

Seibold and Ercegovae (1950) state that, in a dilution of 1 in 3000, a dose of 0·000003 gm. per 1 gm. body weight protected rats infected with *T.equiperdum*, while the controls died in 3 days.

Van Hoof *et al.* (1940) determined the portective power of suramin in guinea-pigs against infection with *T.gambiense* transmitted by tsetse flies. According to them, 0·025 gm. per kilo. of the drug would protect for at least 58 days, while infective flies could be made less infective by feeding them on guinea-pigs treated with suramin.

Van Hoof *et al.* (1947) give their findings on the relative efficacy of the 3 commonly used drugs, namely, tryparsamide, tartar emetic and suramin for laboratory animals infected with *T. brucei.* The curative dosage of these 3 drugs was found by them to be 0·3 gm. per kilo., 0·05 mg. (3 injections) per kilo. and 0·05 gm. per kilo. respectively. Surfen C was ineffective.

II. SURAMIN IN ANIMAL TRYPANOSOMIASIS

The records of treatment of animal trypanosomiasis with suramin may be grouped in accordance with the form of trypanosomiasis against which the treament was applied.

A. In surra (T. evansi *infection*)

It will be convenient to consider the records of treatment of this form of trypanosomiasis in accordance with the species of animal in which the disease occurred.

(*a*) *Equine surra*

The two areas in which suramin has been extensively used in combating surra of horses are the Dutch East Indies with its adjacent territories and the Philippines. Work carried out in each of these areas is briefly described below.

(*1*) *In the Dutch East Indies and neighbouring territories*

In view of the high incidence of surra in the Dutch East Indies, the experience of workers in that country on the use of suramin in dealing with this disease assumes special significance. Judging from all accounts, suramin would appear to be regarded there as the drug of choice, both as a curative and as a preventive. It is stated by Kuneman (1935) that in East Java surra was detected in 124 horses, 364 buffaloes and 152 oxen, in 1934 alone.

In an article covering nearly 80 pages, Rodenwaldt and Douwes (1922) have embodied the results of an extensive series of trials carried out with suramin in the treatment of equine surra in the Dutch East Indies. Their experience with this drug was generally one of disappointment, particularly due to the fact that the toxic dose of the drug was found by them to be very near the curative, although previous workers had claimed that the ratio between the two doses was 11: 167. They describe the toxic effects of the drug as consisting primarily of urticaria and pododermatitis, while small doses not only either failed to kill the parasites or prevent relapses, but also produced cumulative intoxication. They further disclosed the fact that they communicated these results to the Bayer firm in a confidential report, but the firm refused to give credence to them. Rodenwaldt and Douwes state having treated 21 horses; recovery was obtained in only 3 of these. Particulars of doses of the drug given to 6 unsuccessful cases were as follows: (*i*) Single dose of 5 gm.; (*ii*) single dose of 7·95 gm.; (*iii*) 15 gm. in doses of 0·5 gm. every other day; (*iv*) 11 gm. in doses of 1 gm. every other day, relapse and given 3·7 gm. ; (*v*) 7 gm. in doses of

0·25 gm. every other day, discontinued due to toxic symptoms, resumed after 14 days until a total of 15 gm. given ; (6) 1·7 gm., relapse after some days, then 1·8 gm., relapse again, re-treated 4 times unsuccessfully with the same dose. All 6 horses either died or were killed *in extremis*. The horses weighed 122 kilo., 373 kilo., 442 kilo., 325 kilo., 126 kilo., and 170 kilo. respectively. The 3 successful cases were given single dose of 5 gm., single dose of 5 gm. and 10 gm. in doses of 0·5 gm. every other day, the horses weighing 216 kilo., 175 kilo. and 166 kilo. respectively. They found that a dose of 3 gm. conferred protection on a 85 kilo., horse for 2½ months. They recognize the possibility of cure in early stages of the disease either by a single dose of 5 gm. per 200 kilo., or by a series of 0·5 gm. doses every other day, until a total of 10 or 15 gm. has been administered. For prophylactic use, they recommend a dose of 1 gm. per 150 kilo. repeated after 4 weeks.

Baermann (1922), working in the Malay Archipelago, would appear to have been the earliest to test the efficacy of suramin on a large scale against surra in horses. He used the drug in a 10 per cent solution and found that the curative dose for guinea-pigs, rabits and sheep were 0·025 gm., 0·1 to 0·2 gm. and 1·5 gm. respectively. For healthy horses, the dose was 3 gm. per 50 kilo., limited to a total of 10 gm., for the administration of higher doses was likely to result in pododermatitis, oedema and other toxic symptoms. Horses in good condition may, according to him, be given 2·5 gm. per kilo. He deprecates the use of small preliminary doses. For preventing relapses, he recommends 2 or 3 doses of 1 to 1·5 gm. per 50 kilo. at intervals of 25 to 35 days. According to Baermann, a suitable course of treatment may result in the recovery of 52 to 75 per cent of the cases. In a later communication, Baermann (1923) recommends for curative purposes 4 to 6 doses of 5 to 6 gm. against natural surra in equines.

In the annual report of the Agricultural Department, Java, for 1921, Bubberman (1922) expresses doubt as to the real therapeutic value of suramin. In the report for 1923, the drug is stated to possess some curative value only for recent cases of equine surra. Special mention is made by him of the toxic effects of the drug, which, in the case of horses, lent itself for use in doses not higher than ½ gm. per 150 to 200 kilo., on alternate days, until a total of 10 gm. was reached. A dose of 1 gm., however, proved effective for prophylactic purposes. The drug yielded better results in bovines.

In an article almost as lengthy as that of Rodenwaldt and Douwes (*ante*), Bubberman Douwes and Van Bergen (1925) record their experience on the use of suramin in the treatment of equine surra in the Dutch East Indies. Their findings were largely in accord with those of the other 2 workers, the drug having been found by them to be valueless in advanced cases and in relapses. They experimented with 2 lines of treatment, namely, with fractional doses and with large doses. Their main findings were as follows. *A. With fractional doses.* (*i*) Suramin combined with tartar emetic. (*a*) ½ gm. of each alternately with 1-day intervals; 4 horses. (*b*) ½ gm. of each alternately with 2-day intervals; 4 horses. (*c*) 1 gm. of each alternately with 4-day intervals; 6 horses. (*d*) 1 gm. of each alternately with 7-day intervals; 8 horses. Of the 22 horses thus treated, 8—mostly advanced cases—died during treatment, 6 were cured, 1 relapsed, 1 died of other causes, 3 died after relapse and 3 of nervous symptoms, apparently of surra. The recovered cases had received, except one, 10 gm. of each drug. Of the 6 horses that died, 5 received less than 6 gm. and 1 (a large horse) received 10 gm. (*ii*) Suramin combined with atoxyl given to 3 horses; of these, 1—an advanced case— died shortly after, 1 died of other causes and the third, which was in the early stages of the disease, recovered after receiving 10 gm. in 2 months. *B. Treatment with large doses.* (*i*) Suramin combined with atoxyl, a single dose of 3 gm. of each, with an interval of 8 to 14 days between the two drugs; 3 early and 2 advanced cases; 1 died of intoxication, 1 glandered and 3 cured. (*ii*) Suramin combined with tartar emetic ; 4 horses; all died, due apparently to drastic action of emetic. Four others given emetic after suramin; 3 recovered, the fourth was destroyed on account of suramin intoxication. (*iii*) Simultaneous intravenous treatment with suramin and atoxyl; 3 early cases cured after symptoms of suramin intoxication. In 5 other cases—all advanced—the simultaneous treatment was followed 124 hours later with tartar emetic at 1 gm. per 100 kilo.; 2 recovered and 3 died. Bubberman and Douwes consider a combination of slightly toxic doses of suramin and atoxyl as the most promising line of treatment.

Bakker (1925), working in Padang (Sumatra), found a suitable prophylactic dose against equine surra to be 1 gm. repeated at monthly intervals during an outbreak of the disease, this statement being based on observations made by him on 321 horses. Heck (1935) in the Dutch East Indies also gives 1 gm. as a satisfactory preventive dose, his view being based on observations on as many as 500 horses. Bakker (1925) reports having obtained recoveries in 2 out of 4 horses either with a combination of suramin, tartar emetic and atoxyl or of suramin and tartar emetic only; the total quantity of each drug given by him was 4 to 9·2 gm., 3 to 4 gm. and 3 to 5½ gm., in doses of 1 to 4 gm., ½ to 1 gm. and 2½ to 3 gm. respectively. In a thesis submitted by Bakker in 1940, he amplifies and supplements his earlier observations. According to him, suramin is almost invariably effective when used in early stages of equine or bovine surra. He recommends a dose of 5 gm. for buffaloes, 3 to 5 gm. for Java cattle and 4 gm. for horses weighing about 175 kilo. The best results were obtained by him when the drug was used in combination with atoxyl or tartar emetic. A monthly injection of 1 gm. suramin per 100 kilo. was found by him satisfactory as a prophylactic for horses.

Nieschulz (1931) records having cured an experimental case of equine surra by intravenous injection of suramin at 20 mgm. per kilo., combined with intramuscular injection of 30 mgm. of 'Hochst 4002' at 30 mgm. per kilo. The drugs were administered 4 weeks after infection and were repeated 2 days later, although the first injections had cleared the circulation. This treatment was also successfully applied in case of surra in a dog.

Kraneveld and Djaenoedin (1938, 1948) found that there was no evidence of immunity in horses that had been treated for surra 3 months previously with an intravenous dose of 3 gm. of suramin in combination with a subcutaneous dose of 3 gm. of arsocoll (atoxyl). The same authors (1949*a*) infected 3 horses with *T. evansi* intraspinally. One, which was left as control, died after 9 days. The second was treated immediately after infection with suramin and arsocoll as above, but it died on the 64th day with the trypanosomes in crebrospinal fluid; they were not present in the peripheral circulation. Curiously, the third which was given a similar treatment on the 5th day progressed to recovery. The work of Kraneveld and Djaenoedin throws some light on the question of production of resistant strains of *T. evansi* as a result of using subcurative doses of suramin. According to them, there is a possibility of production of such strains in already infected horses when, as is usually done, 1-gm. doses of suramin are used prophylactically. They, therefore, used 3-gm. doses. Such resistant strains, however, are readily amenable to the action of larger doses of the drug. That this is so is illustrated by the following experiment. Twenty four horses, artificially infected with surra, were treated with 1 or more doses of 1 gm. of suramin. They were then given curative doses of 3 gm. of suramin combined with 3 gm. of arsocoll.; 4 only out of these relapsed, but they recovered after a second dose. According to Huber (1940), there is evidence that *T. evansi* in the Dutch East Indies is liable to become drug-fast.

(2) *In the Philippine Islands*

Yutuc (1934, 1936, 1941) made extensive studies on the value of suramin in the treatment of equine surra in the Philippines. The results of these studies are embodied in a series of 3 articles covering nearly 58 pages and they may be considered in some detail. In the first paper of the series, he (1934) states having used the drug at a dose of 10 mgm. per kilo. in 10 per cent solution. A total of 3 doses at 4-day intervals were used. The treatment yielded cure in 6 out of 10 natural cases and also in 3 horses artificially infected with the disease. A noteworthy observation recorded by him was that, when animals were experimentally infected, the trypanosomes appeared in the cerebrospinal fluid after a period of 6 to 22 days. The smaller number illustrates the speed with which surra progresses in the equine species. In his second paper of this series, Yutuc (1936) describes experiments in which 10 horses, artificially infected with *T. evansi*, were treated unsuccessfully by the intravenous-intrathecal method (Edwards, 1928) when the parasites had appeared in the cerebrospinal fluid. Three of these horses were then given repeated intravenous injections, simultaneously, of etharsenol (*p*-3-hydroxy-ethylamine phenyl arsenic acid) and suramin, in doses of 1·77 to 2·24 gm. and 0·59 to 1·49 gm. respectively. All the 3 animals recovered and were in good condition when seen 1 to 2 years later. In his third paper of the series, Yutuc gives an account of a large series of experiments which were undertaken by him for reasons stated by him to be as follows: 'In view of the conflicting findings reported by various investigators on the merit of the combined treatment—that is, naganol with atoxyl on one hand, and naganol and tartar emetic on the other, an attempt was made to determine the value of each combination in non-lethal but slightly toxic doses'. Yutuc's findings were as follows:

(*i*) *With simultaneous injections of suramin and atoxyl.* Seven horses were treated and all died, these results being at variance with those recorded by Bubberman, Douwes and Van Bergen (*ante*) in the Dutch East Indies. Yutuc considers it possible that this is attributable to a difference in virulence of the strains. Nevertheless, Yutuc describes treatment with this combination as 'little good'.

(*ii*) *With simultaneous injections of suramin and sodium antimony tartrate.* Six experimentally infected animals were treated and 2 recovered; 3 of the others died of drug intoxication and 1 died shortly after the tartrate injection and this, according to him, lends support to the view advanced by Edwards (1926) that tartar emetic is contra-indicated in massive infections, for the capillaries are likely to be occluded by the suddenly killed trypanosomes. The combination was also tested on 3 natural cases and recovery was obtained only in 1. Of the 2 animals that did not recover, 1 failed to do so 'principally due to the comparatively small doses of the drug', while the other succumbed 'to a complicating double pneumonia'. Yutuc later used the suramin-emetic combination in the treatment of natural surra in equines in 2 different districts in British North Borneo and the results were as follows: *District 1.* Ten horses treated and 2 reported alive after over a year *District 2.* Ninety horses treated and 61 alive after over a year. Yutuc suggests that the disparity in results obtained in the 2 districts might be due to the fact that in District 1 the doses of suramin and tartar emetic were 2 gm. and 1·3 gm., while in District 2 the doses were 3 gm. and 1·5 gm. respectively. The disparity, according to him, might also be due to the fact that, as the disease was first observed in District 1, 'it is not unlikely that most of the surra-treated animals were in a more advanced stage than those of the other district'. In his review of the work carried out in the Philippines and British North Borneo, Yutuc (1940) enumerates a number of drugs tested by him in the treatment of surra and he states that suramin combinations were found by him to be the best, particularly as prophylactic.

Topacio (1938) in the Philippines also found a course of alternate injections of suramin and tartar emetic fairly satisfactory in the treatment of both artificial and natural surra in equines. The course consisted of 6 injections, at 5 to 7-day intervals, of—(1) 40 c.c. fouadin (tervalent antimony compounds of pyrocatechol sodium disulphonate) subcutaneously and 2 gm. suramin; (2) 1 gm. tartar emetic; (3) 2 gm. suramin; (4) 1 gm. tartar emetic; (5) 2 gm. suramin; (6) 1 gm. tartar emetic. Both suramin and tartar emetic were administered intravenously. The horses weighed from 300 to 500 lb. Topacio records having obtained 6 recoveries out of 11 artifically infected and 44 out of 50 natural cases by adopting this schedule of treatment. Yutuc (1938), working in British Borneo, used 20 to 30 c.c. of a 10 per cent solution of suramin followed, at an interval of 24 hours, by 50 c. c. of a 2 per cent solution of tartar emetic.

De Jesus *et al.* (1949) claim to have evolved an effective and economical method of using suramin in combination with tartar emetic for the treatment of equine surra. They recommend an initial intravenous injection of the drug at 1 gm. per 200 lb. body weight in a 1 per cent aqueous solution, this being followed a week later by the intravenous injection, at 3 to 4-day intervals, of tartar emetic at 0·5 gm. per 400 lb. body weight in a 2 per cent normal saline solution. The entire course occupies about 5 to 7 weeks. According to them, the function of suramin is possibly to sensitize the parasites to phagocytosis and also make them more susceptible to the action of tartar emetic, which, being maintained at a low trypanocidal strength, does not produce the toxic symptoms usually associated with this drug.

In India, Edwards (1926) was the earliest to test the value of suramin in the treatment of experimental surra in equines. The important conclusion that emerged from the results of the use of suramin by Edwards was that, when it was used at 7·5 gm. per 1000 lb. body weight, it conferred protection on horses for 22 days, but it failed to protect for 2 months when used in doses of 10 gm. per 1000 lb. body weight. In order to deal with trypanosomes that had lodged themselves in the cerebrospinal fluid and were, therefore, potentially responsible for causing relapses, Edwards devised the system of combined intravenous-intrathecal medication to which a reference has been made earlier. It is, however, worthy of note that in some instances horses artificially infected with surra parasites by the intrathecal route were cured when suramin was given intravenously. A suitable curative dose for intravenous injection was found by him to be 5 gm. per 1000 lb. body weight in a 10 per cent aqueous solution. According to Edwards, the relatively heavy molecules of suramin constitutes a highly desirable property of the drug, for it is due to these heavy molecules that the drug is slowly excreted and, therefore, retained for a relatively long time in the circulating blood in trypanocidal concentration. The combined intravenous-intrathecal treatment, although effective, proved impracticable under field conditions, and it was later found that a single intravenous injection of 5 to 8 gm. of the drug per 1000 lb. body weight was generally effective in the treatment of equine surra (Sen, 1933).

Since the introduction of suramin by Edwards in the treatment of surra in this country, the drug has been used in different States of India as a routine measure for combating both equine and bovine trypanosomiasis. For example, Farbrother, Director of Veterinary Services, Bombay, writing in one of his annual reports of his experience in Bombay State and Sind, mentions having obtained recovery in a large proportion of the 62 cases of surra dealt with by his department during the year 1934-35. Gopalkrishnan (1942) in Assam records having treated, without a single relapse, 300 cases of surra in ponies, during the period of 1935-39, with suramin given intravenously in 2 injections. The first dose was 50 ml. of a 4 per cent solution and the second 50 ml. of a 6 per cent solution given after an interval of

6 days. Kulkarni (1946), working in Baroda State in India, states that in dealing with equine surra he used suramin in 2 injections of 5·0 gm. each per 1000 lb. body weight, at intervals of 4 to 6 weeks. He claims to have obtained recoveries in 132 out of 165 horses and to have failed only in 1 out of 61 horses treated prophylactically.

Suramin also appears to be the drug used in the routine treatment of equine surra in several other countries. The salient features of the records of work in these countries are indicated below.

Broudin (1927) claims considerable success in dealing with equine surra in Indo-China, by the use of a combination of suramin and sulfursenol. He obtained recovery in the case of: (*a*) An English throughbred by subjecting it to a course of treatment as follows. 10·9 gm. '914', 3·6 gm. '914', 5 gm. suramin and 7·5 gm. suramin, at intervals of 2, 2, 7 and 7 weeks respectively. The animal, however, died from undetermined causes. (*b*) Natural surra in a stallion by treating it with 2 gm. each of sulfursenol and suramin. (*c*) Natural surra in 5 horses, weighing 100 to 250 kilo., by treating them with 2 gm. sulfursenol and 3 gm. suramin. (*d*) An experimentally infected horse by treating it with 1 gm. sulfursenol and 1·5 gm. suramin. (*e*) 5 experimentally infected horses by treating them with 2 gm. sulfursenol and 3 gm. suramin. He also reports having cured 3 experimental cases of canine surra with 8 cgm. sulfursenol and 12 cgm. suramin. Similar results are reported by Broudin *et al.* (1928), who refer to the use of the mixture as 'la technique de choix' in the treatment of experimental surra in equines.

Yakimoff *et al.* (1929) claim to have cured a case of equine surra with suramin at a dose of 0·0125 gm. per kilo. in a 25 per cent aqueous solution. Chevret *et al.* (1937) in Syria report having successfully treated cases of equine surra with suramin administered at 0·01 gm. per kilo.

Jacotot (1936), from his experience in Indo-China, favours the use of a succession of small doses in the treatment of equine surra. According to him, even a dose of 0·75 gm. of suramin is sufficient to cure horses that have been recently infected. As a routine treatment, he recommends an initial dose of 1 to 2 gm. suramin, followed by doses of 2 gm., 2 or 3 times at intervals of 1 week. He found that weekly doses of 2·5 gm. of suramin protected animals for 5 months.

Lionnet (1941) in Mauritius states that he used suramin successfully in the treatment of equine, surra, while soamin, and tartar emetic respectively were used by him for the treatment of surra and *T. vivax* infection in bovines.

In view of the generally favourable reports from differnt countries concerning the value of suramin in the treatment of equine surra, it is somewhat remarkable that Granouillet and Do-Van-Vien (1938) in Cochin China found the drug altogether ineffective against trypanosomiasis (apparently surra) in all the three common species of domestic animals, namely, horses, cattle and dogs.

Equines being particularly vulnerable to *T. evansi* infection, it is of the utmost importance that treatment with suramin in these animals should be applied in the earliest possible stages of the disease. Zijp (1929) considers that in equine surra suramin is only effective when it is used in the initial stages.

It was occasionally noticed by the compiler of the present monograph that the use of antrypol—one of the well known equivalents of suramin—in the treatment of experimental surra in equines resulted in the production of toxic symptoms which manifested themselves *inter alia* in the form of oedema of dependent parts and redness of the mucous membranes. This would also appear to be the experience of Jennings and Jones (1948), who state that they used antrypol in the treatment of U.S. and Chinese army horses and mules in Burma and China during the Second Great War with marked toxic effects. Samples of the drug were later tested on 7 healthy horses in America and 5 of these either died or were killed *in extremis* after they had shown symptoms of intoxication.

Reference may also be made here to the few records of the use of suramin in the treatment of the disease known as murrina (*T. hippicum* infection) of horses. Clark *et al.* (1933)* record a recovery of 31·3 per cent with a combination of suramin and tartar emetic in the treatment of this disease in Panama. They found that 'tryparsamide did not kill the infection even over a long course of treatment in a horse and a mule'. Eakins (1935) obtained as many as 80 per cent cures in murrina by 3 weekly intravenous injections of a solution of 1 gm. tartar emetic and 8 gm. suramin in 400 c.c. water. The dosage injected varied from 50 c.c. for an animal weighing 100 lb. to 20. c.c. for an animal of 600 lb. or over.

A record of the treatment of *T. marocanum* infection in horses with suramin may also be mentioned here. Velu, Barotte and Lavier (1925) failed to cure *T. marocanum* infection in a horse by 5 gm. suramin 6 weeks after the infection. Seven months later, when the animal showed symptoms of wasting, it was treated with 5 and 2 gm. of the drug at an interval of 24 hours but the disease progressed to a fatal issue.

(*b*) *Surra of cattle*

Although in India *T. evansi* is generally regarded as innocuous for the bovine species occasional instances occur when the parasite assumes the status of a virulent variant for these animals. The degree of virulence is illustrated by a series of failures reported by Mahajan (1934) in obtaining recoveries in a severe form of bovine surra in which the affected animals died within 3 to 4 hours of onset of the infection. As trypanosomes were not present in the circulating blood of these animals, it is possible that death was due to what is now known as 'cryptic' forms of the parasites. Mahajan had presumably to deal with an unusually virulent strain of *T. evansi*, for Iyer and Sarwar (1935), working at Karnal in the Punjab, claim to have usually obtained recoveries in cattle, including buffaloes, by the intravenous injection of 1 to 1·5 gm. of the drug. Sen (1936) in India found the drug effective in experimental cases of bovine surra; he used a dosage of 5 gm. per 1000 lb. body weight. As remarked by Sarwar (1937), suramin, in view of its high cost, is hardly suitable for use in the treatment of cattle in India. Mahajan himself (1939) found suramin satisfactory in the initial stage of bovine surra, but not when the disease had advanced. He was, however, more successful with suramin in the treatment of equine surra, for he obtained recovery in 21 out of 28 equines by the intravenous injection of 50 c.c. of a 10 per cent solution per 100 (*sic*) lb. body weight. Mudaliar and Ray (1947) found that in the treatment of bovine surra in India tartar emetic could be used as successfully as suramin, provided the infection was not massive.

Douwes (1923) in the Dutch East Indies formed a favourable view concerning the efficacy of suramin for surra of ruminants, for he obtained cure in 7 out of 11 infected buffaloes treated with single injections of 3 to 5 gm. of the drug in a 5 per cent solution. He also records having cured 3 Bengal oxen affected with surra.

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*These workers regard, on the authority of Darling, murrina as indentical with both *T. hippicum* : infection and derrengadera. On the authority of Wenyon, they state *T. hippicum and T. venezuelense* to be races of *T. evansi.*

The record of work carried out by Lagas (1926) in the control of bovine surra in Sumatra is of special interest, for his work was confined to the disease as it affected buffaloes only. Lagas states that he treated 'prophylactically' 811 buffaloes, of which 64 were carriers but in good condition, 94 showed marked clinical symptoms only and 42 showed both clinical symptoms and trypanosomes in the blood. The results of treatment with suramin carried out by him—so far as it can be gathered from a summarized report of his work —were as follows: Forty-nine of the buffaloes died from various causes, 9 continued to show the parasites in blood, but 8 of these recovered after a second dose of 2 to 4 gm.; the others appear to have effected a recovery after a single dose of the drug. Lagas recommends a dose of 3 gm. of suramin for the treatment of surra in buffaloes.

Kasai and Akazawa (1927) found that a dose of 1 gm. of suramin per 100 kilo. protected Formosan water buffalo calves against surra for 4 months and 2 gm. per kilo. protected horses for 3½ months. Kazanskii (1939) recommends suramin as a prophylactic against surra in horses, cows, asses and dogs.

Jacotot and Nguyen-Dinh-Lam (1935) claim to have cured 3 severe cases of bovine surra, one treated with suramin combined with sulfursenol and serum from an experimentally infected calf, the second with suramin combined with sulfursenol and the third with suramin only. Suramin was given intramuscularly in 'an initial dose of 3 gm., being followed after a week's interval, with doses of 1·8 gm. given at intervals of 3 days.' These workers thus used more than 1 dose of suramin, but it has not been possible to ascertain exact particulars, the article having been seen only in abstract.

In very recent infections of bovines with natural surra, even a subcutaneous dose of 2 gm. suramin in 20 c.c. water would appear to be effective, as shown by Jauffret (1939) in Indo-China. For equine surra he recommends a dose of 1·5 to 2 gm. per 150 kilo., to be repeated after an interval of 8 days.

Richters (1943) recommends the use of suramin dissolved in antimosan for the treatment of all forms of trypanosomiasis in valuable animals, while for large-scale treatment of bovine surra, he advocates weekly injections of tartar emetic in doses not exceeding 0·5 gm. He suggests, for prophylaxis, suramin injection every 4 weeks, although he refers to statements having been made by certain workers that germanin (the brand of Suramin for human use) confers protection on man for 2 to 3 months.

(c) *Surra of camels*

Suramin has proved almost a specific in the treatment of surra of camels, particularly in Sudan and Northern India, where these animals are commonly used as beasts of burden. There are also a few records of the drug having been used with notable success in combating camel surra in Russian Turkestan and in Syria, as will be seen from what follows.

Herzog and Lavier (1923) would appear to have been the earliest to test the efficacy of suramin in a dromedary naturally infected with '*detab*'. A dose of 4 gm. of the drug in a 10 per cent solution cured the animal.

Ilowaisky and Zeiss (1923) tested suramin for its efficacy against '*su-auru*' in 2 camels. An intravenous injection of 3 gm. cured one animal, but in the other relapse occurred after 1 month and a second dose of 3 gm. had to be given to effect a cure.

Yakimoff *et al.* (1927*a*) record having used suramin successfully in the Ural region as a prophylactic against surra in a group of 53 camels. They used the drug in a dose of 5 to 10 gm. None of these animals contracted surra during a post-treatment observation period of 3 months. Seven of a group of 38 controls continued to show trypanosomes. Yakimoff *et al.* (1927*b*) give 5 to 10 gm. of suramin as a good protective dose against camel surra. The duration of protection is stated by them to be 3 months ('Naganol vor einer Ansteckung im haufe von drei Monaten vorgenommen'). Yakimoff himself (1927) states that a dose of 5 to 10 c.c. of a 15 per cent solution of suramin conferred protection on camels for 3 months.

For the treatment of '*su-auru*' to which a reference has been made earlier, Emelin (1928) and Zeiss (1928) consider suramin as the best and they recommend a dose of 4 to 6 gm., while 5 to 10 gm. is considered by them as a suitable prophylatic dose. Zeiss (1937) himself used suramin with success in the treatment of camel surra. He recommends the employment of the drug in doses ranging from 4 to 10 gm., intravenously.

Morcos (1931) lays down the following routine treatment for camel surra: 3 and 4 gm. respectively by the intravenous route at an interval of a week followed by another dose of 4 gm.

Suramin has proved consistently satisfactory in the treatment of camel trypanosomiasis in Sudan. Knowles (1925) records having used it successfully in every one of a group of 6 camels infected with *T. soudanense*. Two of these were treated each with 3 doses of 6 gm., 1 with 8-gm. doses and 3 with a single dose of 10 gm. He writes: 'One dose of 10 grammes of Bayer 205 appears to be an efficient dose for the cure of trypanosomiasis of camel.' Knowles (1927*b*) also records having used suramin successfully at a dose of 10 gm. in 10 per cent solution in the treatment of 60 cases of camel trypanosomiasis. He tested the efficacy of the combination of suramin and tartar emetic in the treatment of 12 others and was successful in 11, the remaining one having died of another infection. In combined treatment, he recommends the use of suramin first when the animal concerned shows massive infection, for otherwise intoxication may result. According to him, the advantage of the use of a combination of the two drugs consists in the reduced cost. In his report of the Veterinary Department, Sudan, for 1927, Bennett (1928) mentions having found a dose of 10 gm. of suramin as a cure in 100 per cent cases of surra in camels. He expresses doubt as to the value of this drug as a prophylactic. Bennett (1933) states that he initially used a dose of 10 gm. but later found a dose of 4 gm. equally effective. He (1934) records having had only 2 failures in treating as many as 4,200 camels with the dose of 4 gm. during a period of 3 years. In another report, Bennett (1933*b*) claims that not a single relapse occurred in nearly 1,000 cases of camel trypanosomiasis treated with 4 gm. of suramin each. In a much later report, however, he (1936*c*) recommends a dose of 5 gm. According to him, the administration of the drug is followed by an immunity for 2 months.

Kennedy (1933), also working in Sudan, states that, since the adoption of routine treatment of camel surra with the single 4-gm. dose of suramin, the losses in camels from all causes have been reduced from 41 to less than 8 per cent.

Soulie *et al.* (1936), referring to camel trypanosomiasis (apparently *T. evansi* infection) in Syria, records having used suramin successfully at a dose of 0·01 gm. per kilo when it was combined with trypoxyl at 0·01 gm. per kilo., tartar emetic at 0·104 gm. per kilo. and anthiomaline at 0·003 gm. per kilo. It is of interest that Pigoury and Bernard (1938), also working in Syria, likewise found it more effective to combine 3 gm. suramin with 3 gm. trypoxyl or 1 gm. anthiomaline in the treatment of camel trypanosomiasis.

Alongi and Balboni (1935), working in Tripoli, however, report suramin having been found valueless as a prophylactic against camel surra. Of other unfavourable reports about the drug, mention may be made of one by Evans (1946), who states that the form of suramin known as antrypol proved ineffective in several cases of camel trypanosomiasis in Sudan.

Another record of failure of suramin in controlling camel surra is that of Receveur (1938), who found the intravenous injection of suramin in doses of 5 to 7 gm. ineffective in this disease—apparently *T. evansi* infection— in the neighbourhood of Lake Chad (near Sudan).

In India, Williams (1930) claims to have used a dose of 4 gm. suramin successfully in the treatment of camel surra. Taylor (1934) in the Punjab records what would appear to be remarkably good results from the use of a combination of suramin and tartar emetic in the treatment of trypanosomiasis of both horses and camels. He shares with Knowles (*ante*) the view that the main advantage of such combination is to reduce the cost which the use of suramin alone would otherwise entail. Taylor used both drugs intravenously at intervals of 4 to 5 days, the actual schedule of treatment being in the following order: first injection of 1 per cent suramin, 2 injections of tartar emetic of 1 per cent and 1·5 per cent respectively, 1·5 per cent suramin, 2 injections of tartar emetic of 2 per cent and 2·5 per cent respectively, 1 injection of 2 per cent suramin; the dosage of drugs used for all injections was 100 c.c. Taylor obtained nearly 88 per cent recoveries amongst 5,692 horses and nearly 87 per cent recoveries amongst 2,135 camels in 5 years by adopting the schedule described above. He, however, considers that a single injection of suramin would have given equally good results as found by Bennett in Sudan, but that a long course ensures some rest for the camels and this, according to him, is highly beneficial for the animals.

Judging from the records of observations in India and the Sudan over a number of years, it may be concluded that suramin has proved to be a most valuable drug for camel trypanosomiasis. The extent to which the drug has gained in popularity is illustrated by the fact that, during 1935 alone, some 10,000 cases of camel trypanosomiasis were treated with this drug in Sudan (Williams, 1936). To the records quoted above of the successful use of suramin in the treatment of camel surra, may be added one by Tremlett (1944), who appears to have used it with success in combating this disease in British Military Administration in Italian Somaliland.

(*d*) *Surra of dogs*

There are on record very few instances of suramin having been employed in the treatment of surra in dogs. Blanchard, Broudin and Borel (1927) mention the instance of a surra (*T. annamense*)-infected dog treated by them, with what would appear to be complete success, with a dose of 0·5 cgm. of suramin dissolved in 5 c.c. of water. The animal, however, developed transient nervous symptoms and was destroyed by its owner without any examination having been made.

The intramuscular injection of suramin dissolved in neo-trepol suspended in isotonic water was found by Jacotot (1929) effective in the treatment of surra, neo-trepol being a metalloid bismuth precipitated from tartro-bismuthate of sodium and potassium. Recovery was obtained by him in 2 dogs injected with 12 cgm. and 6 cgm. suramin in 0·8 c.c. and 0·8 c.c. neo-trepol per 5 and 8 kilo., body weight respectively. Jacotot also obtained cure in 2 horses injected with 1·5 gm. and 0·75 gm. suramin in 10 and 5 c.c. neo-trepol respecively and in 2 buffaloes injected with 1·5 gm. suramin in 10 c.c. neo-trepol. The advantages of neo-trepol are not clear and a large number of workers have reported successful results with suramin alone or in combination with tartar emetic in the treatment of canine surra. The intravenous injection of suramin alone was found generally effective by Van Duln and Coultre (1932). It may be mentioned here that Launoy (1933) found that suramin, at a dose of 4 cgm. per kilo., protected cats for 5 months against *T. annamense* infection. Mention may also be made here of a case of artificial *T. marocanum* infection in dogs which Lavier and Velu (1923) cured by the intravenous injection of 40 to 50 cgm. of suramin. They, however, stress the importance of treatment well before ('précocement') the animal is much anaemic and 'cachatisé'.

In regard to trypanosomiasis of pigs (*T. simae* infection), it was found by Lloverol and Philippe (1943) in French Guinea to be resistant not only to suramin, but also to atoxyl, tryparsamide and tartar emetic. On the other hand, Kraneveld and Mansjoer (1947) record having treated successfully 3 cases of porcine surra by the intravenous injection of suramin in 2-gm. doses. The animals weighed 150, 110 and 100 kilo., respectively.

B. In nagana (mainly T. congolense, T. vivax *and* T. brucei *infections)*

In 1922, that is, soon after the introduction of suramin in the field of chemotherapy Klein and Fischer (1924) published a series of important exprimental observations on the therapeutic and prophylactic efficacy of the drug for both man and animals. The results of interest from the veterinary point of view are summarized here, the species of trypanosome concerned in each case being, it would appear, *T. brucei*. (1) *Glossina*-infected small ox received subcutaneously. 7·5 gm. suramin between 5-7 IV found still infected when tested biologically on 8· V; (2) *Glossina* infected small ox received 12 gm. drug between 8-24·V; found still infected when biologically tested on 28. VI; (3) Another small ox received 14 gm. drug between 14·25. III; found negative for parasites 'up to the time of writing'. (4) Calf dosed with 5 gm. drug; resisted infection when tested by being subjected to bites of *Glossina* 14 days later. The same calf was dosed with 2 gm. subcutaneously on 11. III and again on 12. III; infected when subjected to bites of *Glossina* from 12-19. IV; (5) Ox dosed with 10 gm. between 12-22. III; not infected when subjected to bites of *Glossina* from 22. III-to 2-IV. The gradual separation ('allmählich Ausscheidung') of suramin from the blood is referred to by these workers as its special advantage. In a manuscript report submitted by Klein to the British Colonial Office, he expressed dissatisfaction from the use of suramin prophylactically in cattle. In a communication published by him in 1931, Klein declared suramin valueless in *T. rhodesiense* infection in cattle. Mayer (1922), however, cured *T. rhodesiense* infection in 2 goats by administering the drug by the oral route, the total quantity of drug used in each case would appear to have been unusually large, being about 50 gm. in 1 case spread over about 9 days and 20 gm. in the other.

The results obtained by Berg (1925) in the curative and prophylactic treatment of nagana in cattle with suramin appear to have been far from satisfactory, in spite of the fact that the doses used by him were heavy. All of a batch of 12 oxen weighing from 225 to 230 kilo. died when they were kept in a fly area for 17 to 31 days after being dosed with a total of 10 to 52 gm. of the drug. Twelve further animals were dosed with 10 to 30 gm. and 5 days later were exposed in a fly belt; after another 6 and 10 days they received 10 to 30 gm. and 2 to 3 gm. (twice) of the drug respectively. Eight of the 12 animals died of toxic effects, 1 survived but became infected and the remaining 3 resisted infection, so that, according to Berg, the therapeutic dose of suramin was near the toxic. In another experiment, he dosed intravenously 16 animals with suramin alone, 3 with tartar emetic alone and 13 with a mixture of the 2 drugs, while 16 animals were left as controls. The 2 drugs were used in doses of 2·5 to 10 gm. and 1·5 gm., respectively. All the 48 animals were then kept exposed in a fly belt during December to May and the treatment was repeated every fortnight during this period. About 6 of the 19 animals treated with single drugs and 9 of the 13 treated with both drugs escaped infection. All the 16 controls became infected. Collier (1926) has discussed the rationale of the apparent greater efficacy of a combination of suramin and tartar emetic than that of either drug used alone, but a consideration of these theoretical implications would be outside the scope of the present monograph.

Hornby and Burns (1926) tested the possibility of keeping a group of 10 cattle free from infection in fly belts by injecting them fortnightly, according to Berg's method, with a combination of suramin and tartar emetic. Three of them died within 7 months, 1 reached an advanced stage of the disease, the remaining 6, although not sterilized, were in working condition. He also gave 10 others fortnightly injections of 1 gm. of tartar emetic alone. Two of these died within 7 months, the others continued to remain infected but were workable. Hornby (1938 *b*) found *T. brucei* infection in cattle particularly amenable to treatment with some of the well-known trypanocidal drugs, such as suramin, '314' and Surfen C.

The results obtained by Van Saceghem (1924*b*, *c*) from the use of suramin in the treatment of various forms of animal trypanosomiasis were far from satisfactory. He found that 15 gm. of suramin per 100 kilo. body weight was fatal for cattle. The drug also proved valueless against *T. vivax* infection in doses of 5 gm. per 100 kilo. He found it of little or no value against equine surra, doubtfully effective against dourine and mal de caderas, while in *T. congolense* infection it sterilized the circulation for 4 weeks only. In another paper, Van Saceghem (1925*c*) reports having obtained only a week's sterilization in 4 cattle infected with *T. cazalboui* and *T. congolense* when the drug was administered at about 2·4 gm. per kilo. He (1925 *d*) further observed that a dose of 5 gm. per 100 kilo. did not prevent simultaneous infection or when the animal was subjected to test inoculation 15 days later.

In the treatment of *T. congolense* infection of cattle, Van den Branden (1926*d*) obtained results which were very different from those recorded by Van Saceghem (*ante*), for he found that native cattle could be rid of this infection when they were treated with 3 doses of the drug, each of 1·5 to 2 gm., at intervals of 2 or 3 days. The drug was, however, found by him to be ineffective against *T. dimorphon*.

Van der Elst, Noekermar and Missal (1929) found that the only successful treatment for *T. dimorphon* infection in cattle was a combination of suramin and tartar emetic (*cf.* Collier, 1926, *ante*). They record having failed to cure an animal with tartar emetic, suramin, tryparsamide or tartar emetic combined with trypoxyl and another animal with tartar emetic combined with tryparsamide, tartar emetic combined with suramin or with suramin alone. Recovery was obtained in both animals when they were treated with 3 1-gm. doses of emetic and 2·5-gm. doses of suramin (Poulenc), the two drugs having been used on alternate days. They carried out in all 22 experiments and the form of suramin used by them was moranyl, which these workers evidently regard as being therapeutically different from 'Bayer 205'.

Sicé and Malbrant (1929) attempted to protect a batch of 12 oxen with suramin during their journey to a fly belt over a period which would appear to have been 1 month. Eight oxen were kept as controls. The drug was used subcutaneously at a dose of 3 to 5 gm. according to size. Some casualties occurred on the way when the animals became infected and were treated. Fourteen animals reached destination, and of these, 3 had recieved 1 and 4 had received 2 injections; 7 were kept as controls. No fresh infection occurred in the first group, but there was 1 in the second group and 1 in the controls. During the entire course of the journey, the number of animals infected was : 3 out of 7 (42·8 per cent) in the 1-injection group, 1 out of 5 (20 per cent) in the 2-injection group and 4 out of 8 (50 per cent) in the controls. The dosage of suramin worked out on an average at about 5 to 7 cgm. per kilo.

While the records of work in the treatment of nagana deal for the most part with cattle, suramin has occasionally been used in Africa in controlling nagana in equines. Tarantino (1934), working in Italian Somaliland, found suramin valueless in *T. congolense* infection in horses, but in *T. brucei* infection the drug gave encouraging results. Bennett (1936*d*) has produced evidence that suramin possesses a 'suppressing' value against *T. brucei* and *T. congolense* infections in horses. Five animals, which were found to be harbouring the trypanosomes, were kept in fly belts where horses were not known to survive for more than a few weeks. They were given a 2-gm. dose of suramin every 10 to 12 days and were thereby maintained in a fit condition, one of them having received the treatment for 5 years. Bennett, however, concedes that 'the procedure would be expensive and laborious and would only be justifiable in very exceptional circumstances'. Bennett makes the statement that, while a dose lower than 2 gm. will not prevent a relapse, this dose was the maximum that could be tolerated by the animals.

Vaccari (1942*a*,*b*) found suramin satisfactory in *T. brucei* infection in both horses and bovines if given within 15 days of infection at 2 gm. per 100 kilo., divided into 3 intravenous injections spread over 14 days. De Sousa (1947) treated *T. brucei* infection in 4 donkeys and 6 cattle each with 3 intravenous injections of 3 gm. of suramin at intervals of 5 to 7 days. Recovery was obtained in all the 10 animals, but 2 of the donkeys relapsed.

Suramin would appear to have been seldom used in the treatment of nagana in dogs. Van Saceghem (1925 *c*) reports having cured experimental *T. brucei* infection in a dog with a single intravenous injection of 50 cgm. of suramin. On the other hand, he failed to obtain recovery in a mule naturally infected with this parasite. This animal relapsed a month after receiving 3 gm. of the drug, when it was given a dose of 4 gm. It relapsed again after 2 months and died from undetermined causes 10 days after receiving another 4 gm. Carmichael (1933) records having obtained recoveries in 4 experimental cases of nagana in dogs by the intravenous injection of suramin at 0·015 gm. per kilo. repeated on the 3rd and 10th days.

C. In dourine (T. equiperdum *infection*)

Pfeiler would appear to have been one of the earliest to test suramin in natural dourine. He treated some 116 cases and was unsuccessful in a large proportion of them ('in einer Anzahl von Fällen'). He (1922*a*) tested the value of suramin as a prophylactic against dourine by injecting intravenously 2 gm. of the drug into a stallion; it proved resistant when infected with *T. equiperdum* 2 days later, but the animal showed symptoms of wasting and swelling of the testicles, indicating that no real protection was conferred on the animal. He advises the injection of a dose of 3 gm. of the drug during the covering season, followed, 8 days later, by another 3 gm. and a further dose of 3 gm. 4 to 6 weeks later. Pfeiler (1922 *b*) discusses the reasons of apparent failure of suramin in some horses naturally infected with dourine.

Pataki (1923), in treating natural cases of dourine, observed that 0·01 gm. per kilo. was curative, but produced inflammation of the coronets ('akute Pododermatitis meist auf allen Extremitäten harvorgerufen') and neurosis of the skin.

Balozet, Lavier and Velu (1923) record having cured a case of dourine by the intravenous injection of 1 gm., 4 gm., and 2 gm. of suramin on the first, second and fourth days respectively. The animal had proved resistant to 28 gm. of atoxyl given in 6 intravenous injections.

According to Ruppert (1923), failure to cure dourine with suramin is generally due to incorrect dosage. That the drug possesses a real protective value against dourine is shown by the fact that he found that a dose of 0·4 gm. of the drug per kilo. protected rabbits for 6 months against *T. equiperdum.*

Velu, Barotte and Lavier (1925) treated 6 natural cases of dourine, each with a total of 7 gm. in 2 doses of 5 and 2 gm. at 24-hour interval; 3 showed toxic symptoms and 1 died; 2 effected a doubtful recovery and 1 was cured. According to Barotte (1926), suramin is not superior to combined emetic and atoxyl in dourine; he considers the use of this combination to be the 'traitement classique'.

For donkey stallions, Cordier and Menager (1933) found that suramin was not only ineffective as a prophylactic against dourine, an intravenous injection of the drug at 2 to 5 gm. per 300-350 kilo. was followed by oedema, skin eruptions and other toxic symptoms. In a later paper, Cordier *et al.* (1934), although stating that suramin possesses doubtful therapeutic value for dourine, recommend its use as a prophylactic in this condition.

Velu and Zottner (1934, 1935*a*,*b*) recommend a subcutaneous injection of suramin at about 0·0035 gm. per kilo. every 10 days as a preventive against dourine. They found suramin sometimes causing grave accidents to particularly sensitive animals. They refer to the use of suramin as 'attractive' but they counsel improvement in the technique of using it ('si le principe de la methode est séduisant, le technique a encore besoin de être amélioree'). The same workers, in collaboration with Morod (1934), had earlier concluded that, for prophylaxis against dourine, suramin should be used in small repeated doses. Balozet (1945) apparently accepts Velu and Zottner's view concerning the use of suramin in 1-gm. doses every 10 days as a prophylactic against dourine. He found its prophylactic use successful in horses kept in stables but open to infection. According to him, it is possible to obtain a complete recovery ('guérison totale, definitive') either with suramin or Ciuca's method of treating with novarsenobenzol. Eyraud (1934) also found suramin to be a satisfactory prophylactic in a dose of 0·006 gm. per kilo. When used in this dosage, it conferred protection on stallions against dourine for 15 days.

Velu, Zottner and Belle (1938) repudiate the objections advanced by Cordier and Menager (*ante*) that suramin is liable to cause untoward symptoms in horses prophylactically treated with this drug against dourine. They observed that no such untoward symptoms were produced in Moorish donkeys even when 36 injections of the drug were given to them at 10-day intervals.

Suramin has enjoyed widespread popularity in Europe and other countries where it has been regarded almost as a specific for dourine. Thus, Carpano (1939) in Albania found both atoxyl and suramin satisfactory for prophylaxis against the disease. Good results ('ganz günstige Heilungziffer') are also reported by Endrejat (1939) in the treatment of dourine with suramin in Iran. Of a total of 13 cases treated by him, he obtained recoveries in as many as 11. He used a total of 11 gm. intravenously for each animal, the initial dose being 4 to 5 gm. and the subsequent doses 2 to 4 gm., at intervals of about 1 week. Saurat (1946), referring to conditions in France, states that dourine has been found there to be amenable to treatment with suramin.

An anonymous contributor (in *Mitt. Landw. Turkei*, 1941) recommends for the treatment of dourine a total of 1 gm. of suramin per 50 kilo., this quantity being divided into 3 parts administered at weekly intervals. As an alternative, he recommends the use of 4 gm. of suramin dissolved in 40 ml. antimosan and administered intravenously twice at 3-day interval, followed by 40 ml. antimosan 3 days later. According to Ullmann (1949), however, suramin in combination with antimosan causes a temporary relapse in carrier animals and refers to the possibility of this phenomenon being utilized for the diagnosis of the disease in doubtful cases.

D. In mal de caderas (T. equinum *infection*)

Although mal de caderas is not known to occur in India, the extent to which this disease responds to the action of suramin is of some interest to veterinary workers in this country inasmuch as mal de caderas affects the equine species, which has been most subjected to treatment with suramin against surra.

Migone (1922), working in South America, found that 3 doses of suramin at 2, 3 and 4 gm., given at intervals of about 1 week, were curative for mal de caderas. Migone and Osuna (1922*a,b*) also make a similar statement on the basis of observations on 107 horses. They (1922*a*) treated 219 horses prophylactically and found that a dose of 2 gm. conferred protection for 6 days. The injection of 70 c.c. of a 10 per cent solution of the drug to 2 horses weighing 250 to 300 kilo. resulted in the production of toxic symptoms characterized by swelling of the lips, pharynx, hoofs and testicles and, later, by eczema round the anus and other parts. On the other hand, 2 horses that weighed 150 to 200 kilo., and received 20 c.c. of the solution, showed no such symptoms. Migone (1922) gives the prophylactic dose of suramin against mal de caderas as 2 gm. He reports having obtained complete sterilization in horses with 1 c.c. per kilo. body weight of a 40 per cent solution of the drug combined with urotropine.

Schmidt and De Oliviera (1924) record having cured all of a batch of 40 small animals by suramin, while cured dogs proved refractory to infection up to 114 days. They consider 79 gm., given in 3 doses at weekly intervals, as sufficient for curative purposes. Schmidt himself (1924) has brought forward evidence to show that the use of small doses of the drug in the treatment of mal de caderas does not result in the production of drug-fast strains. He used doses of 0·3 gm. up to a total of 2·5 to 3 gm. and this sterilized the circulation for 10 days. A complete cure was effected after a single dose of 0·5 gm.

In the case of derrengadera (*T. venezuelense* infection, which is considered synonymous with mal de caderas), Tejera (1924) found that 2 doses of 3 gm. of suramin given at 10-day interval was effective, but not a single dose of 4 gm. He recommends a dose of 4 gm. repeated every 6 weeks for prophylactic use against the disease. On the other hand, Morgan (1939) states that suramin has no curative effect for the disease, although it is capable of prolonging the life of the affected animal. He, however, records having successfully prevented animals from contracting the infection by prophylactically injecting them with 1-gm. doses at 3-monthly intervals.

Dios (1925*a, b*) found suramin satisfactory in mal de caderas. He(1925*c*), however, failed to cure a horse with a total of 17 gm. of suramin spread over 4 months. He considers that long intervals between doses are inadvisable. Hetzel (1942), describing his 25 years' experience in the use of suramin, states that, while the drug has proved of value in the treatment of early stages of infections with *T. brucei*, *T. evansi*, *T. equinum* and *T. equiperdum*, it has been found particularly valuable for prophylactic use against the last named parasite. He recommends dissolving the drug in antimosan for use against this infection.

III. Suramin in Human Trypanosomiasis

It would be convenient to consider the records of use of suramin in human trypanosomiasis (*T. gambiense* and *T. rhodesiense* infections) under 3 different heads, namely, (*a*) use of suramin alone; (*b*) use of suramin in combination with other drugs; and (*c*) prophylactic use of suramin.

(*a*) *Use of suramin alone*

'Bayer 205' in its 2 forms, germanin and naganol, has been an extensively used drug for a number of years in the treatment of human and animal trypanosomiasis, but it does not appear to be known to this day why the manufacturers recommend the germanin brand for medical use and naganol brand for veterinary use. In India, its equivalent now in common use is antrypol. A large mass of valuable information on the nature of its trypanocidal action and also on the question of suitable dosages for use in human medicine has emerged in the course of the employment of this drug by medical workers. As this information is likely to prove of use to veterinary workers, it is briefly dealt with in this monograph. As will be seen from what follows, failures to obtain cure with suramin when used in the early stages of human trypanosomiasis, have been few. It is more than probable that the percentage of failures would have been smaller if adequate measures were taken in all cases to conform to the method of using the drug, as prescribed by accredited authorities on the subject. Mayer (1922*b*) has stressed 14 points in order to derive the maximum benefit from suramin. The more important of these are the use of sterile and fresh aqueous 10 per cent solution; due regard to the possibility of kidney changes, as evidenced by the appearance of albuminuria; desirability of using small doses at long intervals; a dose of 0·5 gm. followed by 1 gm. of suramin after 24 to 48 hours in man and 1 gm. per 50 kilo. in 14 days, with a maximum single dose of 7 gm. in horses.

In 1920, that is, about 3 years after the introduction of suramin for the treatment of human trypanosomiasis, Yorke reported having cured a case of Rhodesian sleeping sickness by the intravenous injection of 0·5 gm. of suramin in 5 per cent solution, followed by a second dose of 1 gm. in 10 per cent solution. The blood became completely free from parasites in 7 days.

An instance of the efficacy of suramin recorded in the early days of its use is provided by the recovery, reported by Muhlens and Menk (1921), of a European patient after receiving 0·5 gm. of the drug followed by 2 successive daily doses of 1 gm. each. The patient had proved refractory to tartar emetic and atoxyl. In a manuscript report, Kleine and Fischer (Abstr. in *Trop. Dis. Bull.*, 19, 322; 1922) claim to have obtained what have been called 'biblical cures' in 28 out of 30 cases treated with this drug; it is stated that 16 of these had reached an advanced stage.

Low and Manson-Bahr (1923) used suramin in 7 human patients, with recovery in each of them. The drug was used intravenously in doses of 1 to 2 gm., suitably spaced until a total of 10 gm. had been given.

Hanington (1924) treated 132 early cases of sleeping sickness with suramin and the results are summarized in Table VII.

TABLE VII

Results of Suramin treatment in 132 early cases of sleeping sickness

Number of patients treated	Number of doses used	Number of deaths
25	1—2	6
73	3—5	3
16	6—10	3

The remaining 18 cases were left untreated; death occurred in 4 of these. Hanington refers to the shortness of the course of treatment with suramin as one of its outstanding advantages.

Van den Branden and Van Hoof (1924) give some useful information concerning the relative efficacy of suramin when used (1) intravenously, (2) by the combined intravenous intrathecal method and (3) intravenously combined with the intrathecal injection of serum of suramin-treated patients. The first was found to be the most effective, the second produced alarming symptoms and the third did not prove superior to the first.

The view is widely held that suramin is valueless for the purpose of sterilizing the cerebrospinal system. Letonturier *et al.* (1924*a*) used the drug in doses of 1 gm. at 3 to 7-day intervals, a total of 2 or 3 injections being given. Thirty-five patients, all infected for about a year, were treated. Nine of the 13 with normal cerebrospinal fluid recovered and 4 relapsed. The remaining 22 had developed changes in the cerebrospinal fluid and of these, 11 died and 6 relapsed, without any improvement in the others. In another paper, Letonturier *et al.* (1924*b*) give some impressive illustrations showing the methods of lumbar puncture and examination of blood smears on a mass scale. Chesterman (1924*a*, *b*), on the basis of his extensive experience in this field, likewise found the drug 'frankly disappointing' in cases of cerebrospinal involvement.

A small section of workers report having found suramin not superior to certain other well-known drugs previously in use. Thus, Tanon and Jamot (1925) not only found it valueless in cases of spinal infection but even not better than atoxyl in the first stage of sleeping sickness. In their experience, suramin was effective against *T. rhodesiense* infection, but of doubtful value in *T. gambiense* infection.

Kellersberger (1926) states that he obtained 100 per cent cures when suramin was used in the early stages of sleeping sickness. Halfer (1925) mentions permanent recoveries having been obtained by him in the first stage of the disease by 3 injections of the drug in 1·2-gm. doses spread over a month.

As is the fate with most other trypanocidal drugs, suramin began to show itself ineffective —although admittedly the best of all drugs till then introduced into the field of chemotherapy—when tested by a large number of workers in widely separated localities where sleeping sickness was prevalent. Kinghorn (1925) used it in 30 cases with only 5 survivals. The drug was given in 3 doses of 1 to 2 gm. each at intervals of 10 to 18 days. In the following year, Kellersberger found that the drug was only effective when employed before involvement of the cerebrospinal system and this fact has been fully established in respect of most of the drugs so far used in the treatment of human or animal trypanosomiasis.

A number of workers, particularly French workers, are strongly in favour of replacing suramin by tryparsamide when cerebrospinal infection has set in. It is somewhat paradoxical that a considerable section of workers have expressed the view that tryparsamide is almost ineffective for the purpose of sterilizing the blood circulation, for no explanation appears to have been provided as to why the drug endowed with the property of penetrating the meninges should be incapable of performing the simpler function of clearing the circulating blood.

McLean (1927) states that suramin, although ineffective in advanced cases of sleeping sickness, may produce a temporary recovery, but that this is almost invariably followed by relapse and death. Most workers, as is now well known, recommend tryparsamide as the drug of choice for dealing with cerebrospinal involvement, but according to Corson (1931*a*), both suramin and tryparsamide are ineffective in advanced cases of *T. rhodesiense* infection and also in cases of relapse, and this, he observes, 'is in accordance with clinical experience'.

Van den Branden (1926*c*) has laid down 15 cells per cmm. as the limit up to which cerebrospinal involvement may be allowed to advance for suramin to be capable of producing cure on intravenous injection. According to Kunert (1942), however, a reduction in the cell content of the cerebrospinal fluid is not an indication of progressive recovery, but Kunert's view must be regarded as exceptional, for all workers are agreed that the cerebrospinal picture is the only criterion of prognostic significance. Thus Haveaux (1945) concludes, as a result of his 20 years' experience in the treatment of sleeping sickness in Belgian Congo, that only patients with cell counts of less than 80 may be regarded to be in good condition.

Concerning dosages of suramin, McLean (1926) recommends 4 or more injections of about 1·2 gm. each and he claims to have obtained 14 survivals—during an observation period of 1 year—out of 27 patients treated by him. McLean (1929*b*), on the basis of his experience in the treatment of 52 cases of the Rhodesian form of sleeping sickness, expresses the view that, in simple cases of the disease, a total of 4 gm. of the drug, in 3 to 4 doses spread over a month, may be expected to produce complete recovery, but he considers that for reasons of safety it would be a good plan to inject a total of 8 gm. He (1928*c*) found tryparsamide to be the only drug that held out a prospect of recovery in advanced cases.

One important reason why suramin is regarded as a valuable introduction in the field of chemotherapy is that it lends iteself for effective use in the treatment of cases that are resistant to arsenicals, as is shown by Vaucel and Boisseau (1931*a*) by a tabulated summary of results obtained by them from the use of moranyl (French equivalent of suramin) in the treatment of cases of arseno-resistance.

McLean (1932), working in collaboration with Fairbairn, found that a combination of suramin and tryparsamide was not superior to suramin alone. They record having obtained about 75 per cent recoveries by the use of suramin alone, but they stress the desirability of commencing treatment within a month of appearance of the symptoms of infection. According to them, the drug may be expected to cure 73·5 per cent cases if given within a month of onset of the symptoms, at a weekly dose of 1 gm., until a total of about 5 gm. has been given.

Sicé and Mercier (1934) state that the system of dosage of suramin generally adopted by British workers is the weekly administration of 10 1-gm. doses and that by the Germans 3 or 4 large doses at 2-day intervals. They themselves recommend a dose of 1 gm. at weekly intervals.

The dosages used by McLean (1935) were, in early cases, either 1 gm. every week or 4 dosages of 1 gm. on alternate days, followed by 3 doses of 1·5 gm. each week, the duration of the treatment in both cases being approximately one month. For advanced cases, he recommends an initial course of treatment with suramin, this being followed by 12 weekly injections of 2 to 3 gm. of tryparsamide each.

The extent to which suramin has proved a boon in the work of colonization in Africa is shown by the statement by Gilkes (1936) that the drug has been used as a routine measure to protect workers employed in road construction there from contracting trypanosomiasis. Its efficacy is doubtless attributable to a large extent to the fact that it is slowly excreted. It may be mentioned here that Mayer and Menk (1921) demonstrated the presence of suramin in the urine of treated dogs for 9 days and in the urine of man for 5 days after injection. They (1922*b*) found that the urine and serum of human patients treated with suramin were trypanocidal for about 13 days.

Kunert (1942) makes the statement that, according to his experience, about a third of suramin on intravenous injection enters the cerebrospinal fluid. If this is so, the percentage of recoveries in advanced cases of human trypanosomiasis by the use of suramin alone should have been higher than has been found to be the case by most workers.

Although suramin is considerably safer to use than either arsenicals or antimonials in the treatment of trypanosomiasis, it has not infrequently been found to produce some toxic effects, but, as Fain (1942) points out, the advantages derived from its use far outweigh this defect. The drug is also known to produce albuminuria, but Fairbairn (1944) recommends that, in spite of the appearance of this symptom, the treatment should be proceeded with because, according to his observations, the urine eventually returns to its normal condition.

(*b*) *Use of suramin in combination with other drugs*

The combination of suramin with other drugs, notably tryparsamide, has been claimed by a large number of workers as having yielded superior results than when suramin was used alone, even in the early stages of sleeping sicnkess. Of such workers, special mention may be made of Walravens (1925*a*, *b*) Keevill (1926) and McLean (1928). A combination of the two drugs, on the principle of 'alternation of treatment', should be more effective than either of the two drugs used alone and this in fact has been the view held by Dye (1926) and Manson-Bahr (1931). Curiously, Dye (1926) found that cures were 'spectacular' only when suramin was followed by tryparsamide but that the results were disappointing when the two drugs were used in the reverse order. Walravens (1925*a*) recommends a combination of suramin with arsenicals for the treatment of cases of involvement of the cerebrospinal system, but he considers that arsenicals may as well be replaced by antimonials.

According to De Marqueissac (1932*a*), the ideal treatment for human trypanosomiasis is a combination of suramin and tryparsamide at 0·5 gm. each, repeated every 4 days. He (1933) claims to have employed the combined method of treatment successfully in cases of relapse. The treatment, which was of 12 weeks' duration, comprised weekly injections of the 2 drugs, suramin by the intravenous route during the first 4 weeks and tryparsamide by the subcutaneous route during the next 8 weeks. The first drug was given in 4 successive doses of 0·75, 0·85, 1·0 and 1·5 gm., while the dosage of tryparsamide used was 2·5 gm., per kilo.

Coghorn (1933) has laid down 2 different methods of using a combination of suramin and tryparsamide. One—the prolonged thorough treatment—consists of 5 1-gm weekly doses of suramin, followed by about 7 3-gm. weekly doses with a month's interval between the 2 courses. The other—the short intensive treatment—comprises 3 1-gm. doses of suramin on alternate days, followed by 1 week's interval and then a total of 20 gm. tryparsamide in weekly doses of 3·4 gm. These recommendations are made by him on the basis of his experience in the treatment of 47 cases by the first and 63 cases by the second method. The long course treatment is considered by him to be the better. According to him, 'the prognosis in relapsed patients is death in 3 years of the first relapse in over 97 per cent of cases'.

Dupuy (1936) recommends an injection of sodium hyposulphite prior to subjecting the patient to a course of treatment with heavy doses of tryparsamide—apparently to obviate the possibility of the production of arsenic-resistance.

Lester (1936, 1945) has laid down what he regards as the standard dosage system for the treatment of human trypanosomiasis; this consists of 3 doses of 1 gm. of suramin, followed by 5 2-gm. doses of tryparsamide at 5-day intervals. He, however, recommends a preliminary test dose of 0·2 gm. to obviate the possibility of a higher dose producing fatal symptoms. For cases of relapse, he lays down a course of 3 injections of suramin followed by a course of tryparsamide totalling about 17 gm. A standard treatment, according to Brauwere (1938), is an injection of 1 gm. of suramin, followed by about 14 injections of tryparsamide at the usual dosage.

Harding (1945), working in Sierra Leone, tested the relative efficacy, on 2,713 cases of sleeping sickness, of suramin, tryparsamide, pentamidine and propamidine, used singly or in different combinations. All of the following 3 combinations of suramin and typarsamide-the first in 1-gm. doses and the second in 2-gm. doses—proved successful:

(1) Three doses of suramin followed by 3 to 7 doses of tryparsamide with an interval of about 7 weeks between the first and second and the remainder at 5-day intervals.

(2) Two doses of suramin followed by about 4 to 6 doses of tryparsamide at 5-day intervals.

(3) One dose of suramin followed by 8 or 9 doses of tryparsamide at 5-day intervals.

Harding does not consider the absence of trypanosomes in the blood or improved clinical condition as pointing to recovery: only the picture of cerebrospinal system is, according to him, of prognostic significance.

Eeraerts (1946) is one of a small number of workers who recommend a triple combination of drugs, namely, tartar emetic, suramin and tryparsamide, in the following order: 10 weekly injections of 0·8 gm. of tartar emetic together with 1 gm. of suramin in 10 per cent aqueous solution, this being followed by 10—apparently weekly—injections of tryparsamide at 0·06 gm. per kilo.

McLetchie (1948) in Nigeria used a combination of suramin and tryparsamide in doses of 0·5 and 1·5 gm. respectively, a total of 6 to 20 injections being given at 5-day intervals. Davey (1948) recommends a slightly different combination of the 2 drugs. He states that the standard treatment to be adopted, not only in Nigeria, but also in Sierra Leone and Gambia, is to administer 3 doses of 1 gm. suramin each, followed by 5 2-gm. doses of tryparsamide, all at 5-day intervals. He, however, records having obtained more satisfactory results in Nigeria from 3 doses of a mixture of 1 gm. suramin and 2 gm. tryparsamide, followed by 2 doses of tryparsamide, all at 5-day intervals.

(c) *Prophylactic use of suramin*

As a drug for prophylactic use, suramin would appear to be of very limited value, as is known to be the case with other trypanocidal drugs, antrycide being the only exception by reason of the fact that this drug, in the insoluble form of chloride, is retained for a considerable period in the tissues as a small depot, from where it is slowly liberated into the circulating blood. Nevertheless, suramin has frequently been used with advantage for giving protection against human trypanosomiasis for a limited period of time.

Bossert (1927) used suramin prophylactically in doses of 0·02 to 0·04 gm. per kilo. in 500 individuals, while another 500 were left as controls. After 7 months, 267 of the treated and 313 of the controls were traced. All of the treated were found healthy, but 2·5 per cent of the controls were infected (see, however, Jamot and Chambon, 1930, *infra*).

It may be mentioned in parenthesis that the advantage of suramin as a drug for prophylactic use is, as pointed out by De Brouwere and Lisfranc (1931), is the convenience of administration and the readiness with which it may be dissolved in water for obtaining the suitable concentration.

Mayer (1928) recommends for prophylaxis against sleeping sickness a dose of 1 gm. every 3 months. He states that repetition of the treatment before the expiration of 3 months is useless and rather injurious ('Zwecklos und eher schädlich'). Such recommendations can, however, only be regarded as of value if they are supported by experimental observations on the duration of retention of specific quantities of suramin in trypanocidal concentration in the circulating blood.

Fourche and Ricklin (1928), in their itinerant practice in Belgian Congo, used the drug prophylactically in doses of 1 gm. for adults and 0·3 to 0·75 gm. for children, the doses being repeated at 15 to 30-day intervals. A total of 1,059 individuals received single and 2,042 individuals double injections of suramin. After an interval of 10 months, the percentage of infections in the first group was 0·54 and in the second 0·22, as against 1·00 amongst the non-suraminized. Some of the suraminized, however, had previously been treated with arsenicals and this may have in a few cases contributed to their freedom from infection. Fourche (1931), in collaboration with Hareaux, published another paper on the subject of suramin prophylaxis and in this he made the obviously sound recommendation that prophylactic treatment with suramin should be limited to individuals who are definitely healthy and should not be resorted to in doubtful cases. According to him, 2 injections of the drug may be expected to confer protection for a maximum period of 7 months.

Some figures are given by Arnaud (1929) to show the relationship of the dosage of suramin with its prophylactic efficacy. These are summarized in Table VIII.

TABLE VIII

Prophylactic efficacy of suramin in relation to dosage

Dose per kilo	Number of individuals injected	Percentage of new infections
0·02 gm.	25	0
0·02 to 0·015 gm.	57	7
0·015 to 0·01 gm.	247	17
Less than 0·01 gm.	121	32

The figure of 0·02 works out at an approximately flat dose of 1 gm. (taking the average weight of human patients to be 120 lb.) and this is in conformity with the recommendation made by Mayer (*ante*).

Jamot and Chambon (1930) obtained very different results from the prophylactic use of the drug in an attempt to confirm what had been recorded by Dossert in 1927 (*ante*). They injected 604 individuals with doses of 0·02 to 0·04 gm. and kept 561 as controls. After a period of 8 months, 565 of the treated and 498 of the controls were traced. Among individuals in the first category, 30·7 per cent and among those in the second category 30·5 per cent were found infected.

The results obtained by De Marqueissac (1932*b*) from the prophylactic use of suramin were even more disappointing. A total of 215 individuals were given the drug and 257 were kept as controls. They were re-examined after a period varying from 82 to 123 days. Curiously, 10 per cent of the treated and 7 per cent of the controls were found infected. He, however, records the interesting finding that suramin has the property of sensitizing a race of resistant trypanosomes. Thus, parasites which had been resistant either to tryparsamide or to suramin became sensitive to tryparsamide after it had been acted upon by suramin.

The results of some controlled experiments on human volunteers led Duke (1934) to conclude that the drug was suitable for use prophylactically and that it was more effective against *T. rhodesiense* than against *T. gambiense.* The dose recommended by him is 1 to 1·5 gm. every 3 months. In a paper published by him in 1936, he states that a dose of 2 gm. may be expected to protect for a period of 3 months. Duke (1936) also carried out what would appear to be a unique series of experiments to derive precise information on the prophylactic value of suramin under conditions that were likely to obtain in the field. He made use of volunteers who submitted themselves to the infective bites of tsetse flies after they had received an injection of 1 to 2 gm. of the drug ; one of the volunteers resisted infection for nearly 1 year. Duke considers it likely that a prolonged immunity would be established after receiving repeated infective bites following drug injection.

Orlovitch (1937) gives the results of his observations on a population of 621 prophylactically treated by him at a dose rate of 1 gm. for adults, 0·4 to 0·75 for children and 0·15 to 0·3 gm. for infants. The injections were repeated every 3 months, a total of 3 injections having been thus given. Re-examination of the blood was made before each injection, with negative results in all cases. Eeraertes (1946) used a dose of 1·25 to 1·5 gm. for adults and about 0·03 gm. per kilo. for children. He considers suramin prophylaxis the best for dealing with human trypanosomiasis in endemic areas.

Of other workers who have made notable observations on the value of suramin for human trypanosomiasis, mention may be made of Low and Manson-Bahr (1922) ; Mayer (1922 *c*, review) ; Menk (1923) ; Carpenter (1925); Rawson (1924); Tanon and Jamot (1924); Fischer (1925); Lloyd (1925); Schofield (1925) ; McLean (1926); Stroda and Lopes (1926); Van den Branden (1926, 1927*c*); Cobb (1928); Keevill (1928); Saunders (1928); Shelley (1929); Fisher and Kunert (1930); Lee (1931); Kleine (1931); Paris Eguilaz (1932); Fourche and Marligheim (1936); Millous (1936); Reynes and Trinquier (1938); Sicé (1939); Ciccaldi (1945) and Veatch (1946).

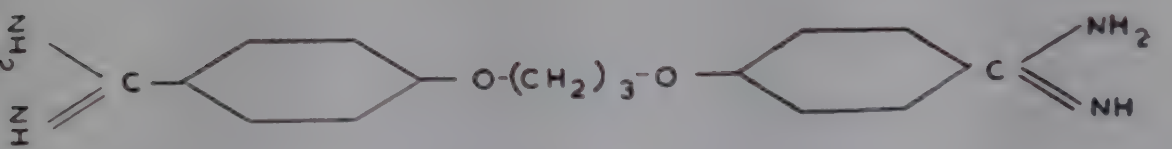
CHAPTER XIII

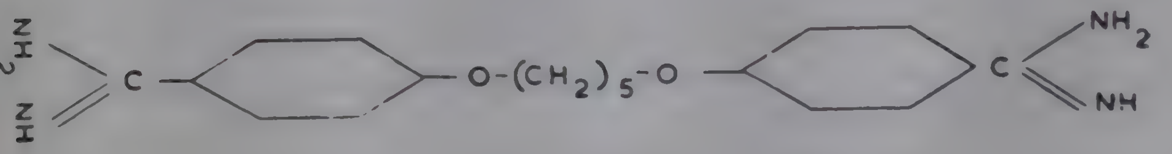
Diamidines

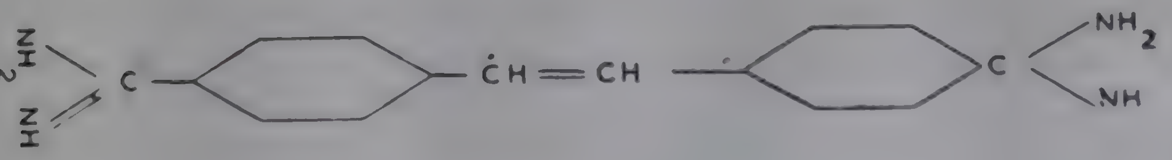
THE diamidines have been fairly extensively used in sleeping sickness, but there are very few records of their having been employed in the treatment of animal trypanosomiasis. In any case, a perusal of the available records of work with diamidines shows that they have been seldom used in the treatment of *T. evansi* infection. The bulk of the work carried out with these drugs in Africa has been in regard to their prophylactic value against trypanosomiasis.

I. LABORATORY TRIALS WITH DIAMIDINES

The possibilities of diamidines as chemotherapeutic agents emerged out of certain findings made by King, Lourie and Yorke (1937, 1938) following upon the discovey made by Jancsó and Jancsó (1935) that synthalin (decamethylene diguanidine) had a direct trypanocidal action. King and his collaborators examined a number of guanidines, isothioureas, amidines and amines with alkyl and alkylene chains and found that undecane diamidine permanently cured laboratory animals infected with *T. rhodesiense*. Lourie and Yorke (1937) had also found that synthalin had a powerful action on *T. equiperdum* and *T. rhodesiense* infections in mice. The following three aromatic diamidines were shown by Lourie and Yorke (1939) to be chemotherapeutically the most active compounds in the series :







In a review of the findings referred to above, Yorke (1940) gives an interesting discussion on the relationship of the chemical configuration of these compounds with their trypanocidal action. He divides the amidine compounds containing the amidine group into 4 classes : compounds linked by a straight-C-linkage, those linked by an-O-linkage, those linked by an-N-linkage and those linked by an-S-linkage, the last having been found to possess little trypanocidal power.

Stilbamidine in doses of 0·05 mg. per 20 gm. mouse was found by Yorke to cause permanent recovery in the majority of mice infected with *T. rhodesiense*, while a total of 1 mgm. given in 2 doses of 0·25 mgm. on consecutive days cured mice infected with *T. congolense*. Way and Chan (1944) found that the tolerated and curative doses of propamidine for mice were 40 mgm. and 5 mgm. per kilo. He experimented with *T. brucei* and *T. equiperdum*.

Following upon the discovery of the high chemotherapeutic value of 4 : 4 diamidine compounds, Fulton and Yorke (1942*a*) investigated a number of others in this series in respect of their action on *T. rhodesiense* and *T. congolense* infections in mice. They found that monomethyl stilbene and dimethyl stilbene were both active against these 2 parasites. They (1942*b*) further observed that solutions of stilbamidine exposed to light for 2 days became more toxic for mice and hence arose the desirability of using a fresh solution each time. Fulton (1943) observed that under such conditions the drug lost in therapeutic value for *T. rhodesiense.* Fulton (1945), working in collaboration with Goodwin on some of these diamidines, found that the increase in the toxicity occurred when they were exposed to light in solution, but not when exposed in a solid state. It is worthy of note that monomethyl derivatives of stilbamidine were similarly affected but not dimethyl derivatives or pentamidine.

Fulton (1944) investigated the prophylactic action of some aromatic diamidines against *T. rhodesiense* and *T. congolense* infections in mice. He found that the injection of these drugs conferred protection for periods up to 2 to 4 weeks and that the effect was proportional to the therapeutic activity of these diamidines. This conclusion would appear to stand to reason, for, *a priori*, the dosage of a drug and the expected duration of its effect should be approximately the same when it is used for therapeutic or prophylactic purpose, although this is not what would appear to be the view held by most workers in this field. One notices that the prophylactic dose is usually much less than that advocated for therapeutic use. On theoretical grounds, it seems reasonable to hold that the curative and prophylactic doses of a drug should not merely be the same but that the prophylactic should exceed the therapeutic dose, with due regard to the therapeutic index, in proportion to the duration of protection which the drug is desired to confer. He makes the general observation that 'the more active diamidines have a definite frank prophylactic effect, which is, however, of limited duration.'

Launoy and Lagodsky (1946*a,b*), from their studies upon the action of penatamidine against *T. brucei* and *T. evansi* infections in rats, found that the curative dose of this drug for *T. brucei* infection was 0·5 mgm. per 100 gm. rat and that the period of protection with this dose was 14 days. This period increased to about 30 days with a dose of 2 or 3 mgm., while 5 mgm. protected for 100 days. The curative dose for *T. evansi* infection was found by them to be 6 mgm. and the dose required to protect for 10 days in 50 per cent of the mice to be 10 to 15 mgm., while 25 mgm. protected against *T. evansi* for 30 days. The larger the dose the longer was the duration of protection. The curative dose of pentamidine for *T. equiperdum* infection in rats was found by Launoy (1946) to be 10 to 12 mgm. per 100 gm., while a dose of 5 gm. per 100 gm. protected for at least 2 months. Launoy and Lagodsky (1946*c*) published some observations which showed that pentamidine was prophylactically far less effective against *T. evansi* than against *T. brucei*. While protection for 100 days was conferred on 100 gm. rats against *T. brucei* by the subcutaneous injection of 0·005 gm. of pentamidine, the much higher dose of 0·025 gm. gave protection aginst *T. evansi* only for a month. The same workers (1946*d*) tested the curative and prophylactic value of pentamidine, when injected subcutaneously into rats, against 3 species of trypanosomes, with the following results. The curative dose of the drug in mgm. per 100 gm. was found to be 0·5 for *T. brucei*, 0·7 to 0·9 for *T. equiperdum* and 7·0 to 9·0 for *T. evansi*. The dosage of this drug required to protect for 25 to 30 days was found to be 2·0, 2·0 to 2·5 and 25·0 mgm. respectively. *T. evansi* thus proved to be by far the most resistant to the drug.

Launoy and Jeanpierre (1948) studied the possibilities of utilizing pentamidine for prophylactic purposes when the drug was given *per os*. Two doses of 10 mgm. per 100 gm. body weight at an interval of 4 days proved ineffective in rats against infection with *T. equiperdum* when tested 24 days after the drug administration, but 3 further doses caused a resistant stage in the animals. Launoy (1948) investigated the possibility of conferring protection on rats infected with *T. gambiense* by the oral administration in gum suspension of a form of diamidinophenoxypentane, but the results were not encouraging. He (1949*a*) also tested a penicillic acid salt of lomidine base but here also the results were not more successful. Launoy (1949*b*) tested the value of both this penicillate and 2 : 2′—methylene-bis oxynaphthoate against *T. gambiense* in rats when these drugs were injected and not administered by the oral route. A dose of 0·08 gm. per 100 gm. rat of the penicillate protected for 40 days, while a dose of 0·035 to 0·05 gm. per 100 gm. rat of the other drug gave protection for 14 to 27 days. As for lomidine, Launoy (1950) found that 100 mgm. of this drug, when injected subcutaneously per kilo., protected for about 6 months in 50 per cent of the animals; 5 mgm. per kilo. for about 25 days; 10 mgm. for 25 to 60 days; and 20 mgm. for more than 2 months. It is of interest that lomidine had been found by him (1947) effective when administered *per os*, 0·15 to 0·3 gm. per 100 gm. of the drug having prevented rats from contracting infection when they were given the parasites 1 to 8 days after drug administration.

II. DIAMIDINES IN ANIMAL TRYPANOSOMIASIS

While diamidines have been used with success in the treatment of sleeping sickness, they have been found by some workers to exert a delayed toxic action in animals. Thus Simmons (1941), in the annual report of the Veterinary Department, Uganda, for 1940, refers to the occurrence of such action, frequently leading to death in 1 to 2 months in cattle treated with these drugs against *T. congolense*, *T. vivax* and *T. brucei* infections. Wilson (1942) in Nyasaland likewise found it valueless against cattle trypanosomiasis. Delayed poisoning was also observed by Daubney and Hudson (1941) in cattle treated with pentamidine and stilbamidine against experimental infection with a virulent strain of *T. congolense* in Kenya. Complete cure was not obtained by any of these drugs. A single dose of 20 mgm. of stilbamidine per kilo. given intravenously sterilized the circulation for 16 days, while 3 doses of 5 mgm. per kilo. at intervals of 7 days, produced only a premunized state in the animals. A premunized state was also brought about by 3 doses of 2·5 mgm. of pentamidine per kilo. at intervals of 14 days, but this drug proved much more toxic than stilbamidine. These workers cite instances of both immediate and delayed toxicity. Hudson (1941) records similar observations in his annual report of the Veterinary Department of Kenya for 1940.

Carmichael and Bell (1943) found stilbamidine almost ineffective against *T. congolense* infection in cattle. They used a 5 per cent solution of the drug intravenously. With single doses of 2·5 mgm. per kilo. and 5·0 mgm per kilo., they obtained sterilization for about 12 days in 4 and 2 cases respectively. Of a group of 6 cattle treated with 10 mgm. per kilo. each, 4 were cured, 1 died and 1 relapsed after 20 days. Of a second group of 6 animals treated with 12·5 mgm. per kilo. each, 2 were cured and developed symptoms of poisoning. Two were treated intramuscularly with 10 mgm. per kilo.; both relapsed after about 15 days.

Carmichael (1943*a*) was more successful with 4:4-diamidino dimethyl stilbene hydrochloride in a few preliminary trials, but in a series of later experiments, he (1943*b*) found the margin of safety between the toxic and the curative dose to be small. He (1944) also tested 5 diamidino phenyl compounds but the results were unsuccessful. Bell (1945) found the protective value of pentamidine against *T. congolense* infection to be also very limited.

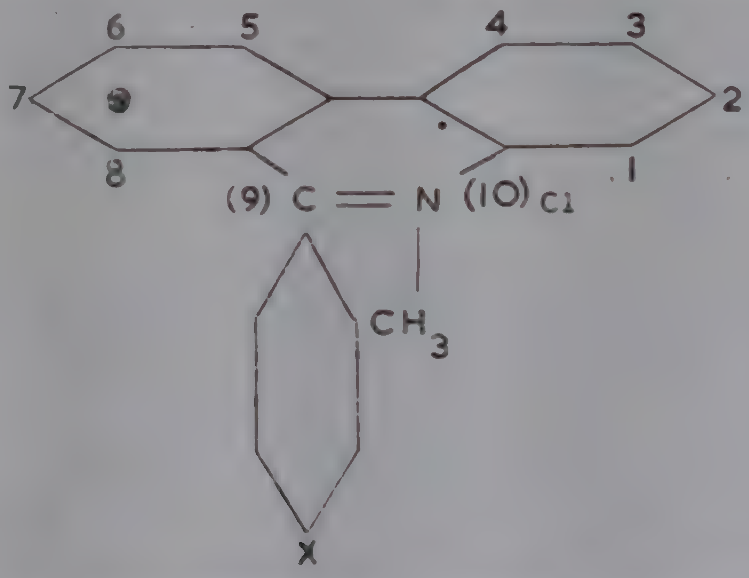
Deom (1949) tested the efficacy of pentamidine, given intramuscularly in the form of a 20 per cent solution in methyl acetamide, against experimental *T. congolense* and *T. vivax* infections in cattle. The drug was tested, at a dose of 1 to 3 ml. per kilo., on 9 cattle and was given either simultaneously with the infected blood or 24 hours to 15 days later. It altered the course of the disease and Deom considers that the results might have been better if the inoculations were repeated.

CHAPTER XIV

Phenanthridinium Compounds

PHENANTHRIDINIUM compounds represent a group of drugs which have been exclusively employed in the treatment of animal trypanosomiasis. The discovery of these compounds constitutes a decisive advance in the control of trypanosomiasis of livestock. It would appear that the outstanding virtue of phenanthridinium compounds, as remarked by Fiennes (1945), is that, whereas other trypanocidal drugs are not curative in the advanced stages of the disease, these drugs are often capable of effecting a cure by repairing and stimulating tissue growth.

A perusal of the available literature shows that the earliest conclusive evidence that phenanthridinium compounds constituted a new type of trypanocidal agents was produced by Browning, Morgan, Robb and Walls (1938). The first two phenanthridinium compounds possessing trypanocidal properties were synthesized by Morgan and Walls and these were as follows :



A. 7 amino-9 (p—aminophenyl)—10 methyl phenanthridinium chloride (NH_2 groups at positions 7 and X).

B. 3(acetylamino)—9 (p—acetylaminophenyl)—10 methyl phenanthridinium chloride ($NH.COCH_3$ groups at positions 3 and X).

Subcutaneous injection of 1 mgm. of A or 2·5 to 5 mgm. of B cured *T. brucei*-infected mice weighing 20 gm. when the drugs were given 24 hours after parasite inoculation, but these doses were about the maximum tolerated. Compound A but not B had also a curative action on *T. congolense* infection. These workers stress the fact that compound A was equally effective against both *T. brucei* and *T. congolense*, for the latter had been found to be usually not affected by agents which were trypanocidal against *T. brucei*. Browning, Browning and Robb (1940) published observations to show that the subcutaneous injection of compound A, in 1/16th to 1/100th of the maximum tolerated dose, cured mice infected with *T. congolense* but, as observed by Browning *et al.* (1938, *supra*), its curative dose—and also the curative dose of B—for *T. brucei* were near the maximum tolerated. Drug A exerted little prophylactic action against *T. congolense*. There were 2 different strains of *T. congolense* used and the dose required to cure infected mice with Drug A was $\frac{1}{15\text{—}20,000}$ gm. in the case of one strain and $\frac{1}{75\text{—}100,000}$ gm. in the case of the other. Browning, Browning and Robb give detailed figures to show the relationship of the dosage with the number of recoveries and some of these figures are reproduced here : (*a*) With the less virulent strain: 0·04 to 0·033 mgm., 4 out of 6 ; 0·02 to 0·017 mgm., 5 out of 6 ; 0·013 to 0·01, 3 out of 3 ; 0·007 mgm. or less, none of a batch of 4 mice. (*b*) With the more virulent strain : 0·1 mgm., 3 out of 4 ; 0·067 to 0·033, 5 out of 5 ; 0·02 to 0·017, 1 out of 4.

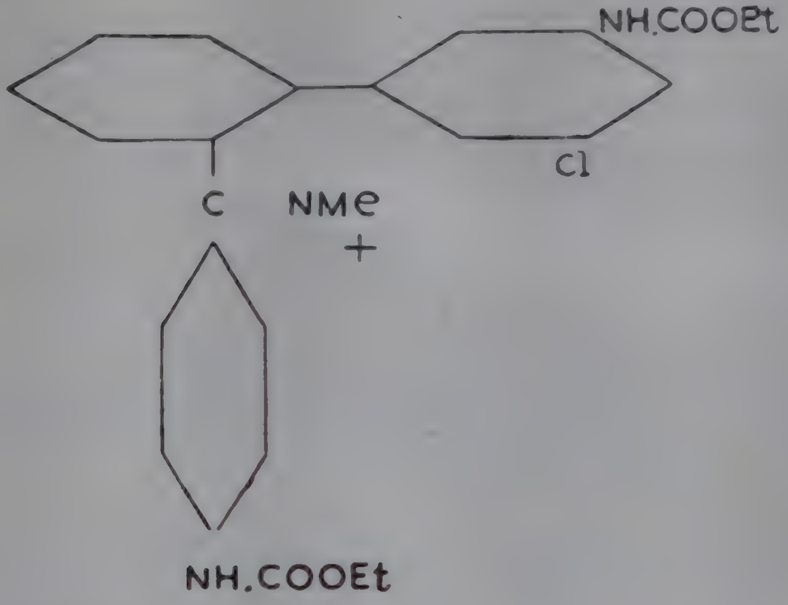
Two well known forms of phenanthridinium compounds have since been commonly used both in laboratory trials and in the treatment of animal trypanosomiasis under field conditions. One of these—the drug referred to as 'A' above is designated—phenidium chloride or 'phenanthridinium 897' or merely '897' or 'phenidium'. The other is 2 : 7—diamino-9-phenyl-10-methylphenanthridinium bromide or 'phenanthridinium 1553' or merely '1553' ; it is also known as dimidium bromide.

In a paper published by them in 1945, Walls, Browning, Calver and Leckie have discussed the relationship between the structure and trypanocidal properties of the phenanthridinium series. They synthesized 22 amino derivatives of 9-phenyl-10-methylphenanthridinium chloride, some of which were found to be effective in mice against *T. brucei* and others against *T. congolense*. The trypanocidal activity of these amines was found to be reduced on acetylation.

In an informative thesis, Calver (1945) has given the results of his studies on the action of phenanthridinium compounds on *T. congolense* infection in mice, this infection, as pointed out by him, having been found more or less resistant to such drugs as arsenicals, suramin, etc. As further stated by him almost the only compounds that had been found to exert some action on this infection were tartar emetic, antimosan and Surfen C. Calver used 2 strains of *T. congolense*, one (strain II) being more virulent than the other (strain I). He uses the term 'acme' to indicate the state of infection when the parasites were abundant, the expression 'acme strain' being accordingly used to mean strain propagated by passage at this stage. The drug tested was principally phenanthridinium '897.' Given subcutaneously, 0·001 gm. per 20 gm. mouse was well tolerated by the mice, but 0·0013 gm. killed 30 per cent of them. It was more effective when given at the 'acme' stage of infection than when given earlier or when the disease had assumed a chronic form. The minimum curative dose per 20 gm. mouse was 0·03 mgm. for strain I and 0·3 mgm. for strain II. There were occasional cases of relapse, but these proved amenable to re-treatment. Mice cured of infection with strain I were immune against this strain during an observation period of 13 months, but only 50 per cent of the mice proved immune against strain II when they were cured of infection with this strain. In both cases the treatment was given at the 'acme' stage. Whether the animal was cured at the chronic or the 'acme' stage, it developed little or no immunity against strains from chronic cases. Animals cured at the chronic stage, however, showed similar immunity to strains from 'acme' infections as did animals cured at the 'acme'. Calver found phenanthridinium '1553' more suitable than phenanthridinium '897'. The latter appeared to be of little prophylactic value.

A noteworthy property of phenanthridinium compounds was that some of them were experimentally shown to be trypanocidal against *T. cruzi*, which had proved to be resistant other chemotherapeutic agents, except 'Bayer 7602' (a 4:6 diamino-quinaldine derivative).

Browning, Calver and Leckie (1946) used 'S1544' (3-carbethoxyamino-9-*p*-carbethoxyaminophenyl-10-methyl-phenanthridinium chloride, and the corresponding methanesulphonate ('S 1577') with considerable success in the treatment of mice infected with *T. cruzi*. The formula of 'S1544' is shown by them as follows :



Seventy animals were treated with the 2 phenanthridinium compounds at the dosage of 2.5 to 3 mgm. per 20 gm. (maximum tolerated), resulting in 13 cures, while 18 showed suppression of the disease. Treatment was more effective when given in the early stages than at the 'acme' and in this respect *T. cruzi* infection responded to the treatment differently from *T. congolense* infection. The superiority of 'S 1544' and 'S 1577' over 'Bayer 7602' was shown by the fact that, out of 23 mice treated with the latter, 2 were cured and 6 showed suppression of the infection. S 1582 (2 : 7-dicarbethoxy-amino-9 : 10-dimethylphenanthridinium methosulphate) was also significantly trypanocidal against *T. cruzi*. Animals in which the infection had been cured or suppressed 'failed to develop an obvious infection' when re-inoculated after a long interval. According to these workers, this showed that the process of immunity also contributed towards recovery.

Wien (1946) compared the chemotherapeutic value of '1553' and '897' and an amidine 'No. 991' (4 : 4-diamidino-X 3—dimethylstilbene dihydrochloride). He confirmed '1553' as being the most effective of these, having a therapeutic index of 152, as compared with 10 of '897' and 12 of '991'.

Launoy and Prieur (1947) carried out a series of control tests on the action of '897', '1553' and an acid sulphate compound of phenanthridinium on small animals infected with different species of trypanosomes. The results obtained with the first two of these were follows:

I. '897'

(*a*) TOXICITY IN MGM. (L.D. 50)

Intravenous. Mice, 0·35; white rats, apparently not tested.

Subcutaneous. Mice, 1·5; white rats, 7·5 (per 100 gm. body weight in each case).

(*b*) TRYPANOCIDAL ACTION

A. Mice

1. *T. brucei*. 0·2 mgm. intravenously and 1 mgm. subcutaneously failed to cure.
2. *T. congolense*. 0·075 mgm. intravenously cured a mouse weighing 20 gm.; 0·1 mgm. subcutaneously cured; controls died.
3. *T. evansi*. Some clearance ('blanchiments') of circulation with 1 mgm. per 20 gm. body weight but relapses occurred regularly.

B. Rats

1. *T. brucei.* Sterilization with 2·5 mgm. per 100 gm. body weight given subcutaneously.

2. *T. equiperdum.* No cure with doses up to 7·5 mgm., this dose having sometimes proved toxic and caused death. Route of injection not stated.

3. *T. congolense.* Sterilization with a dose of 0·5 mgm. given subcutaneously.

II. '1553'

(*a*) TOXICITY IN MGM. (L. D. 50)

Intravenous. Mice, 0·75 ; rats, apparently not tested.

Subcutaneous. Mice, 1 per 20 gm. body weight; rats, between 7·5 and 10 per 100 gm. body weight.

(*b*) TRYPANOCIDAL ACTION

A. Mice

1. *T. brucei.* Intravenously no action with a dose of 0·5 mgm., but 'un blanchiment' of 24 hours with a dose of 0·75 mgm. Subcutaneously 1 mgm. had no action.

2. *T. congolense.* Both subcutaneously and intravenously regular sterilization obtained with 0·075 mgm., the action of the drug being more pronounced when administered by the subcutaneous route. Mice cured by a dose of 0·1 mgm. were found susceptible to a 2nd inoculation of the parasites given 20 days after treatment.

3. *T. evansi.* No cure with subcutaneous dose of 1 mgm.

B. Rats

1. *T. brucei.* Subcutaneously regular sterilization with doses above 0·5 mgm.

2. *T. equiperdum*—No cure subcutaneously with 2·5 mgm. but only 'blanchiment' of 5 days.

3. *T. evansi.* Similar results as with *T. equiperdum.*

Neither of the two drugs had any preventive action against the parasites. It will appear from the results summarized above that the two drugs are particularly effective against *T. congolense* infection.

For some time a misapprehension obtained currency that samples of phenanthridinium '1553' and '897' were variable in their toxic properties—a view also held by Fiennes (1947*a*). Brownlee, Goodwin and Walls (1947) assayed about a dozen samples of '1553' against *T. congolense* infection in mice and found no significant difference in their activity. They were unable to reproduce delayed toxicity either with '1553' or with '897'. After subcutaneous injection, both drugs persisted in the circulation for about 24 hours, but the concentration of '1553' was twice that of '897'. Laboratory experiments carried out by these workers showed that '1553' was about 3 times as active as '897' and they quote Bell and others as having shown that the relative therapeutic effect of the two drugs was of the same order in infected cattle. On the other hand, Baeten (1951) records having found some samples of '897' more trypanocidal than others.

II. PHENANTHRIDINIUM COMPOUNDS IN THE TREATMENT OF ANIMAL TRYPANOSOMIASIS

Of the two common forms of phenanthridinium compounds, namely, '897' and '1553', the later has been more frequently used in the treatment of animal trypanosomiasis. The results obtained with these two forms are shown separately below.

(a) Phenanthridinium '897'

Daubney (1942), workinng in Kenya, used graded doses of phenanthridinium (presumably '897') in the treatment of cattle infected with *T. congolense*. The number of cures obtained by him with different doses were as follows: 0·5 mgm. per kilo., none of a group of 3; 0·75 mgm. per kilo., 1 out of 3; 1 mgm. per kilo., 5 out of 8; 1·5 mgm. per kilo., 1 out of 3; 2 mgm. per kilo., 3 out of 4. Daubney found the drug unsatisfactory for the treatment of relapsed cases, indicating the possibility of drug-fast strains having been produced (*cf.* Carmichael and Bell, 1944*b*). He also found stibophen (the tervalent antimonial sodium antimony *bis*—pyrocatechol—3 : 5—disulphonate) superior in the treatment of *T. congolense* and *T. vivax* infections when the drug was administered at weekly intervals. Surfen C (congasin) was ineffective. The results reported by Daubney in 1943 would appear to be superior to those reported by him in the previous year, for, out of 50 animals treated by him with a single dose per kilo. of 1·5 mgm. of '897', 28 recovered and 22 relapsed, while recovery was obtained with a single injection of the drug in 80 to 90 per cent of infected animals in another herd. Daubney also treated 49 cattle with stibophen, with 21 cures, 8 deaths and 20 relapses, these last occurring within 3 months of drug treatment with 3 doses given at weekly intervals. Twelve of these relapses were successfully treated with single injections of '897'.

Carmichael and Bell (1944*a*), working in Entebbe, Uganda, tested the therapeutic effect of '897' on *T. congolense* infection. Owing to the low solubility of '897' these workers used a 1 per cent solution of the drug. A series of toxicity tests performed by them showed that the maximum non-toxic dose of the drug, administered intravenously, was 3 mgm. per kilo. A preliminary experiment showed 2 mgm.' per kilo. of the drug to be an effective dose for therapeutic use. A herd of 40 (apparently artificially) infected cattle were then divided into 5 groups of 8 each and treated by 5 different routes, viz. (1) intravenous, (2) subcutaneous, (3) intramuscular, (4) combined intravenous and subcutaneous and (5) combined intravenous and intramuscular, 1 mgm. per kilo, being given by each route in the last two. The results of these treatments were respectively as follows (*c*=cured ; *r*=relapsed; *d*=died of intercurrent infection): (1) 3 *c*, 4 *r*, 1 *d*; (2) 2 *c*, 5 *r*, 1*d*; (3) 1*c*, 7*r*; (4) 1*c*, 7*r* ; (5) 4*c*, 4*r*. The 27 relapses were re-treated by a single injection 44 days after the first treatment and recovery was obtained in 22 of them. The other 5 animals showed 3 more relapses and were each time unsuccessfully treated, apparently due to the parasites being drug-fast. Prophylactically used, '897' comferred protection on cattle against *T. congolense* for more than 48 hours. As the workers point out, a serious drawback of the drug is its low solubility, necessitating injection of a large volume of the solution.

De Meza (1944) would also appear to have used phenanthridinium compound in the treat ment of cattle in the Nyasaland Protectorate, but no details are given in his report which has been seen only in abstract. In his report for the following year, De Meza (1945) gives some particulars of the results obtained by him with '897' in the treatment of cattle naturally infected with trypanosomiasis. He found 1 injection of this drug generally effective, the few relapses being successfully treated by a second injection. He further found that stibophen sterilized the circulation for 2 to 3 weeks (*cf.* Daubney, 1942). '897' proved to be distinctly superior to tartar emetic inasmuch as, for curative purposes, 6 injections of the latter drug had to be administered at 5-day intervals.

Mackintosh (1945) reports '897' to be unsatisfactory, but in his paper published in 1947 he records having used both this drug and stibophen with some success in the treatment of *T. congolense* and *T. vivax* infections in cattle in the Uganda Protectorate, where mortality from these infections was very severe, as many as 11,000 cattle having died of trypanosomiasis in one area alone. Later, '1553' proved to be much superior and was used with less than 10 per cent relapses after a single dose in the treatment of *T. congolense* infection, although in animals treated for *T. vivax* infection, 50 per cent relapses occurred several months after the treatment. Severe local reactions were produced when the drug was administered subcutaneously in a 2 per cent solution. It was better borne by the animal when given intramuscularly in a 1 per cent solution. The reported superiority of the intramuscular over the subcutaneous method of administration is, however, contrary to the experience of Carmichael and Bell (1944*a*, *supra*), although Bell in a later paper (1945*a*) reported that, out of a batch of 27 animals that had received the drug subcutaneously, 3 developed sloughs.

Stewart (1948) in Gold Coast Colony found '1553' to be sufficiently effective to be used as a routine measure in the treatment of *T. congolense* and *T. vivax* infections of cattle in that locality, although it was valueless against *T. brucei* infection. Ferreira (1948 also records having found the drug highly effective in the treatment of animal trypanosomiasis in Portuguese Africa. Hobday (1945, 1947) and De Kock (1949) mention having successfully used both '897' and '1553' in the treatment of bovine trypanosomiasis in Northern Rhodesia and Union of South Africa respectively.

(*b*) *Phenanthridinium '1553'*

Carmichael and Bell (1944*b*) published some observations on the use of '1553' in the treatment of *T. congolense* infection in zebu cattle. A 1 per cent solution of the drug could be obtained at room temperature, while a 4 per cent solution could be readily prepared when hot water was used. Its greater solubility made the drug more suitable for use than '897'. They performed 2 experiments. In the first, they infected a group of 20 cattle and when they had developed anaemia and showed loss of condition with numerous trypanosomes in the circulating blood, they were administered a 2 per cent solution of '1553' at a dose of 2 mgm. per kilo. Four animals were injected intramuscularly and 4 intravenously; all the 8 were cured. Twelve were injected subcutaneously and of these, 8 were cured and 4 relapsed between the 13th and 24th day after treatment. These relapses were re-treated successfully with the same dosage given subcutaneously. In the second experiment, 19 chronic cases were treated subcutaneously with varying doses of the drug as follows. (*i*) *At 1 mgm. per kilo.* 4 with 1 per cent and 4 with 2 per cent solution. (*ii*) *At 1·5 mgm. per kilo* 4 with 1 per cent and 4 with 2 per cent solution. (*iii*) *At 2 mgm. per kilo.* 3 with 1 per cent solution. Cure was obtained in all the 19 animals. The authors were unable to investigate the toxicity of '1553' for cattle, but they believe this to be low. In a paper published a year later, Bell gives 0·8 mgm. per kilo. as the minimum curative dose.

Randall and Beveridge (1946) carried out some toxicity tests with '1553' on cattle and found that a dose of 2 mgm. per kilo. was highly toxic under field conditions in Uganda, for nearly all of a batch of 147 cattle developed symptoms of photosensitization after this dosage and 41 of these died from the toxic effects of the drug. In a further note, Randall and Beveridge (1947) give an account of 2 toxicity trials with '1553' carried out on 8 zebu cattle artificially infected with *T. congolense*. The drug was administered in a 2 per cent solution. The resuts obtained in each of the 8 animals are summarized in Table IX (the drug was in each case administered on 25.1):

TABLE IX

Results of toxicity tests with '1553' on cattle

Animal	Dosage in mgm. per kilo.	Route of administration	First symptoms of photosensitization	Results
1	2	Subcutaneous		Died 14.III
2	2	Intramuscular	15.III	Slow recovery
3	3	Subcutaneous	18.III	Do.
4	3	Intramuscular	15.III	Do.
5	4	Subcutaneous	18.III	Died 9.IV
6	4	Subcutaneous		Died 12.III
7	4	Intramuscular	16.III	Destroyed 2.IV
8	5	Subcutaneous	8.III	Destroyed 30.III

All the animals were negative for trypanosomes on blood examination, but each of them suffered a striking loss of weight, as would appear from their weight records shown graphically by the authors. On 28.III., they intramuscularly administered to 3 non-infected cattle 3, 4 and 5 mgm. of the drug per kilo. in a 2 per cent solution. The one that received the smallest dose slowly recovered and the other two died on 6.V. and 13. V. respectively.

Stewart (1946) in Gold Coast tested the efficacy of '1553' under field conditions on 67 cattle, of which 31 were infected with *T. congolense*, 21 with *T. vivax* and 5 with *T. theileri*. In addition, he treated 2 horses infected with *T. vivax*. The drug was used at a dose of 2 mgm. per kilo. administered intravenously. Cure was obtained in all cases without the occurrence of any toxic symptoms. It is to be noted that the dosage used by him was much in excess of 0·8 mgm. per kilo., which had been found by Bell (1945*b*, *supra*) to be the minimum curative dose. He found that the drug had 'an excellent tonic effect.'

Barnett (1946) used '1553' at a dose of 1·25 mgm. per kilo. intramuscularly in the treatment of 6 cross-bred zebu European cattle. Sterilization occurred in each case but 4 of them relapsed and were cured with a 2nd dose of 2 mgm. per kilo. According to Barnett's observation, 'the solution is very irritant'.

Randall and Laws (1947) have published some experimental observations to show that '1553' to be effective is best administered, not in the early stages of *T. congolense* infection, but when the disease has established itself, 'indicating that immunity reactions probably play an essential part in assisting the action of the drug'. Their observations, which were made on a batch of 69 cattle, are summarized in Table X. The drug was used by them in 65 of the animals in doses of 0·8 mgm. to 1·5 mgm. per kilo. in a single injection. In 2 animals, the dose was 0·8 mgm. per kilo. repeated after 14 days; in one, 0·4 mgm. per kilo. repeated after 7 days; and in another, 0·2 mgm. per kilo. repeated 4 times at weekly intervals.

TABLE X

Results of treatment with '1553' in cattle infected with T. congolense

Number of cattle	Interval in days between infection and treatment	Result	
		Cure	Relapse
2	0	—	2
1	2	—	1
1	4	—	1
1	6	1	—
1	8	—	1
8	15	6	2
7	24—30	7	—
4	33	3	1
44	38—117	44	—

Of the 8 relapses, 2 were successfully re-treated with '1553' at 1 mgm. per kilo. and 1 died of acute infection following re-treatment with the drug. The other 5 animals failed to respond to injections of '1553', but in 2 of them the infection appeared eventually 'to have been thrown off spontaneously' and they progressed to recovery. The remaining 3 animals were subjected to a course of treatment with stibophen; 2 recovered and 1 died. The main conclusion drawn by Randall and Laws from these observations is that "'phenanthridinium 1553' is the most valuable drug at present available for the treatment of *T. congolense* infection in cattle" and that "a single dose of 1·0 to 1·5 mgm. per kilo. is very effective in the treatment of fully developed infections and is unlikely to give rise to photosensitization or troublesome local lesions". In the paper by Randall and Beveridge (1947, *supra*), it had been stated that the usual dose of '1553' in Uganda was 5 ml. of a 1 per cent solution per 100 lb. body weight or 1·1 mgm. per kilo. Concerning relapses which occurred in the course of experiments carried out by Randall and Laws, it may be stated that, in the report of Agricultural Department of the Zanzibar Protectorate for 1944, relapses after phenanthridinium are referred to as of more infrequent occurrence than with tartar emetic.

Crawshaw (1947) in the Bechuanaland Protectorate, investigated the prophylacrtic value of '1553' against *T. congolense* and *T. vivax* infections in cattle. The dosage of the drug was 2 mgm. per kilo. administered subcutaneously. Two groups of 3 animals each, after receiving an injection of '1553', were grazed in a fly area for a period of 9 and 14 days respectively. None of the animals developed the infection during an observation period of 206 days in the case of the 1st group and 167 days in the case of the 2nd. Two controls were kept in each of the two experiments and all became infected with *T. congolense* and *T. vivax* and later successfully treated with '1553'. The drug was found to be less active against *T. brucei*.

Crawshaw later carried out an experiment to test the possibility of keeping cattle free from infection in a fly belt by injecting them with 2 mgm. of the drug per kilo. at various intervals. He used a total of 8 animals with 2 controls and his results are summarized in Table XI.

TABLE XI

Results of keeping cattle in fly belts after prophylactic treatment with '1553'

Animal	Number of injections	Interval between njections	Period during which blood smear negative	Interval between last drug injection and first appearance of parasites in blood
1	6	7 days	46 days (died)	Negative
2	6	7 ,,	41 days	Do.
3	4	14 ,,	70 ,,	28 days
4	4	14 ,,	105 ,,	63 ,,
5	3	21 ,,	70 ,,	28 ,,
6	3	21 ,,	70 ,,	28 ,,
7	3	28 ,,	134 ,,	77 ,,
8	3	28 ,,	134 ,,	77 ,,
Control	—	—	49 ,,	—
Control	—	—	42 ,,	—

It would appear from the above table that 3 injections of the drug at intervals of 28 days are capable of conferring protection for 134 days. No difference in the prophylactic action of '1553' was observed whether it was given subcutaneously or intravenously.

Wilson (1947) at Entebbe (Uganda) carried out a series of observations on the curative value of '1553' in artificial *T. congolense* infection in cattle. The animals, which were 54 in number, received the drug intramuscularly or intravenously, but not subcutaneously, and on this point he writes: 'In a small preliminary experiment carried out in February, 1947, the reactions to subcutaneous injections were never serious, but this route was not considered suitable for field use and was not tried in this experiment'. He used the drug in a 3 per cent solution and at the dosages of 1, 1·5 and 2 mgm. per kilo. Three animals were treated intramuscularly and 3 intravenously with each of the three doses, 14, 24 and 34 days after the infective inoculation. Cure was obtained in all cases (observation period 24 weeks), with the exception of 7 animals, particulars of which were as follows: Three relapsed after receiving the drug at 1 mgm. per kilo., 14, 34 and 34 days respectively after the infective inoculation; 3 others, treated 34 days after infection, with 2 mgm., 2 mgm. and 1·5 mgm. per kilo. respectively showed signs of drug intoxication and either died or were destroyed *in extremis*; the 7th recovered after developing symptoms of photosensitization. Wilson records having observed a period of depression, characterized, *inter alia*, by loss of weight in a proportion of the animals 70 to 80 days after they were infected.

Ferreira (1948) found phenanthridinium to be the most effective in the treatment of *T. congolense*, *T. vivax* and *T. brucci* infections in domestic stock, this conclusion being based upon his experience in dealing with neary 334,000 infected animals in Portuguese African territories.

Piat (1948) claims to have used '1553' successfully, in a single intravenous dose of 2 mgm. per kilo., in the treatment of horses and cattle infected with *T. vivax*, although the injections were followed by transitory symptoms of shock.

Wilde (1949) experimentally used both '1553' and '897' in the treatment of zebu cattle infected with *T. congolense* in the Veterinary Research Laboratory, Mpwapwa, Tanganyika Territory. Wilde's main observations are summarized in Table XII.

TABLE XII

Results of treatment of congolense-*infected cattle with '1553' and '897'*

(Wilde, 1949)

Drug	Numer of animals	Dosage	Route of administration	Number of cures	Relapses
'1553'	17 naturally infected	1·5 mgm. per kilo. in 0·5 per cent solution and 0·75 mgm. in 0·25 per cent solution	Subcutaneous or intramuscular. Severe local reaction with former; slight with latter	17	2; recovered on re-treatment with a single dose
'1553'	57 artificially infected	1·0, 1·5 and 2 mgm. per kilo. in 2 per cent solution. Injected between 10 to 30 days of showing trypanosomes	Intravenous or subcutaneous or intramuscular. Local reaction more pronounced with subcutaneous injection	57	None
'897'	5	Do.	Do.	5	Do.

Wilde recommends 1 gm. per kilo. in 2 per cent solution as suitable in the field and he considers the intramuscular route for the administration of the drug as the most satisfactory. He gives a warning regading the instability of solutions of '1553', as shown in an earlier paper by Beakbane and himself (*Vet Rec.*, **60**, 31; 1948).

During 1947-48, Evans (1950) carried out a mass treatment of cattle with '1553'. over an area of 30,000 square miles in Anglo-Egyptian Sudan, where death rate among these animals due to *T.congolense* and *T. vivax* had been estimated to be about 10,000 per month in 1947. He used the drug subcutaneously in a 2 per cent solution at a dose of 1 mgm. per kilo. As a result of this campaign, the death rate was reduced to about 1 per cent after 12 to 18 months. Severe local reactions were, however, produced in 3 to 4 per cent of the animals following the subcutaneous injection.

Deom (1950) used '1553' intramuscularly on 25 cattle in a 1 per cent solution, at a dose of 1 mgm. per kilo. After 5 minutes to 28 days, he inoculated *T. congolense* to 12 of them and *T.vivax* to 11, while 2 animals were given both species of parasites. All resisted infection.

An observation of special interest to workers in India is that recorded by Daudet (1950) on the treatment of 50 cases of equine surra with '1553'. He used the drug intravenously at a dose of 0·002 mgm. per kilo. and recovery was obtained in 48 of them; the remaining 2 died of what would appear to be the toxic effects of the drug.

In an anonymous communication published in the *Bur. Interafr. Tsetse Tryp. Leopoldville* (Abstr. in *Vet. Bull., Weybridge,* **22**, 242; 1952), it is stated that, in an outbreak of trypanosomiasis—evidently of *T.congolense* and *T. vivax* infections—in Southern Rhodesia affecting 1,407 head in a herd of 4, 686 cattle, block treatment was adopted with a 3 per cent solution of dimidium bromide at 1 ml. per 50 lb., intravenously. The number of deaths 2 months after the treatment was only 5, against 121 deaths that had occurred 10 weeks before treatment. Only 3 out of 67 smears were positive for trypanosomes 1 month later, while all of a batch of 24 smears examined 8 months after treatment were negative.

According to Herin (1952), cattle unsuccessfully treated with '1553' show a much greater susceptibility to *T.congolense* infection than to *T. vivax* infection, and in the former case they succumb earlier than they would do otherwise. Relapses are stated by him to be more frequent after unsuccessful treatment in *T.vivax* infection.

Mols and Lenaerts (1950) found dimidium bromide ineffective in 5 piglets infected with *T.simiae* and weighing 15 to 20 kilo. They injected the drug subcutaneously at 5 mgm. per kilo.

Several workers report the occurrence of photosensitization in cattle after treatment with phenanthridinium compounds. As already mentioned, Randall and Beveridge (1946) noticed the occurrence of these toxic symptoms in the course of their trials with '1553'. Wilde (1949) observed that recovery after treatment with '1553' was followed by symptoms of photosensitization, this being attributed by him 'to a build-up of photodynamic substance in the blood after liver dysfunction has been caused by dimidium bromide'. He cites Bell (1947) as having shown that such photosensitization associated with a high mortality may be induced in animals that have received the drug at a dose of 3 mgm. per kilo. According to Randall and Laws (1947), 1·0 to 1·5 mgm. per kilo. of '1553' is not likely to cause photosensitization or local lesions. Baeten (1951) records having observed the occurrence of photosensitization after dimidium chloride in young animals, which, however, were more amenable to permanent cure than adults.

In a report published about 1951 in *Bur. Interafr. Tsetse*, V.P.I.P.T. publication No. 133 0, pp. 7 (Abstr. in *Vet. Bull, Weybridge,* **21**, 609; 1951), Macaulay and Shaw record the results of trials carried out by them with both '897' and '1553' in relation to dosage, possibilities of photosensitization and production of drug-fast strains,but the compiler of this monograph has not been able to obtain particulars of their observations. Photosensitization as a sequela to treatment of cattle with '1553' is also mentioned by Thorold and Plowright (1952) and by Plowright *et. al.* (1952). Burdin and Plowright (1952) observed that the administration of dimidium bromide at 2-3 mg. per kilo. produced liver dysfunction in zebu cattle. The main symptoms of photosenization are described by Plowright *et al.* (1952) as follows: 'hyperaemia of the subepithelial tissues of the muzzle; this became drier than normal and exuded droplets of serum which coagulated into flakes'.

CHAPTER XV

Antrycide

ANTRYCIDE is the trade name given to it by the Imperial Chemical Industries, Ltd., Manchester. In the leading article in *Nature* of Jan. 15, 1949, it is stated that the first public announcement of the discovery of this drug was made at a conference held jointly on the 29th December 1948 by the Colonial Office in England and the Imperial Chemical Industries. Its discovery by Curd and Davey (1949) 'after considerable chemical exploration', as stated by them, is now universally regarded as having registered a decisive advance in the chemotherapy of animal trypanosomiasis.

Curd and Davey tested antrycide chloride against different trypanosome infections in mice with striking success, as would appear from the protocol of these tests reproduced in Table XIII.

TABLE XIII

Results of tests on trypanocidal action of antrycide chloride in mice

Dose (mgm. per kilo.)	*T. rhodesiense* (Tinde)	*T. brucei* (Liverpool)	*T. congolense* (Bhusimbi)	*T. evansi* (India)	*T. evansi* (Sudan)	*T. equiperdum*	*T. equinum*
25	*C*						
12·5	*C*						
5	*C*	*C* and *R*					
2·5		*R*		*C*	*C* and *R*		*C*
1·25			*C*	*C*	*C* and *R*	*C*	*C*
1·0			*C*				
0·5			*C* and *R*	*C* and *R*	*R*	*C*	*C* and *R*
2·25			*R*			*C* and *R*	
0·125			*R* and *DD*				

C = Cure: blood freed from trypanosomes for 28 days.

R = Relapse: blood freed from trypanosomes temporarily, appearing again within 28 days.

D D ± Delayed death: blood not freed from trypanosomes. In all experiments the drug was injected subcutaneously.

It is noteworthy that Curd and Davey mention a Kenya strain of *T.congolense* as having shown marked resistance to the drug, for 'a dose of about 2·5 mgm./kgm. was required to produce consistent cures in mice'.

Curd and Davey tested the prophylactic action of antrycide chloride in mice against a Busimbi strain of *T. congolense* and the results were as given in Table XIV.

TABLE XIV

Results of tests on prophylactic action of antrycide chloride against T. congolense *infection in mice*

Dose (mgm./kgm.)	Interval between dosing and challenging (days)	Result
25	14	10/10 *P*
12·5	14	8/8 *P*
5	14	4/11 *P*. 7/11 *I*
25	28	8/8 *P*
12·5	28	8/8 *P*
5	28	8/8 *I*
12·5	42	2/6 *P*. 4/6 *I*
5	42	8/8 *I*

The above trials were made on mice.

P=Completely protected: no trypanosomes in blood 28 days after challenging with them.

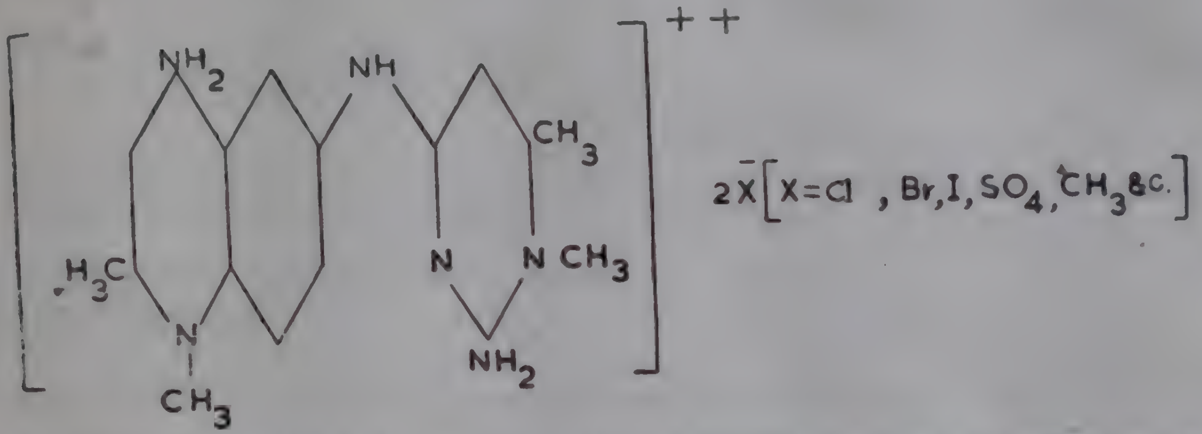
I=Incomplete protection, judged by delayed death as compared with controls. 10/10=10 out of 10 animals tested upon, 4/11=4 out of 11 animals tested upon, etc. Antrycide had no action on *T.cruzi*.

Curd and Davey also report having found antrycide curative for *T.congolense*, *T.vivax* and *T.brucei* infections in cattle; *T.brucei* infection in horses, donkeys and dogs; and *T.evansi* infection in camels. Further, antrycide was found by them to give 'considerable protection' to cattle against infections with *T.congolense* and *T.vivax*. In an article (*Brit. med. J.*, 11 June 1949, pp. 1046-47) based upon an address by Davey, antrycide is stated to confer protection on cattle against trypanosomiasis for at least 4 to 6 months, but this statement failed to receive confirmation.

Relating the circumstances immediately antecedent to the discovery of antrycide, Curd and Davey (1950) stated that among earlier trypanocidal drugs, the amino quinoline derivatives—to which Surfen C belongs—seemed to them to merit exploration. By linking this type with the pyramidine nucleus, which they had previously explored in their investigation of anti-malarial drugs, they eventually developed the class of antrycide salts.

In the communication mentioned above, Curd and Davey (1950) state that antrycide comprises 2 salts, both highly trypanocidal in action but acting in 2 different ways. One is antrycide methylsulphate which is readily soluble in water and the other antrycide chloride which is only slightly soluble in water. In practice, a mixture of the 2 salts—the so-called 'pro-salt'—is injected subcutaneously, so that the soluble portion of the mixture rapidly gets into the circulation, while the suspension of chloride is believed to remain beneath the skin as a small depot from where it is slowly absorbed. The two-fold object of an initial sterilization and the destruction of potential parasites is thus achieved in one operation.

Antrycide is stated by Curd and Davey to be 4-amino-6-(2-amino-6-methylpyramidyl-4-amino) quinaldine-1 : 1—diametho salts. Its chemical constitution is given by them as follows:



Spinks (1950) has published some important observations on the absorption and persistence of antrycide in the circulation. According to him, the solubility of the methylsulphate is about 300 times that of the chloride, and the former is rapidly absorbed, its concentration in the plasma being proportional to the size of the dose injected. On the other hand, the chloride is only partially absorbed, the undissolved portion, as mentioned above, serving as a reservoir of the salt. It is noteworthy that the concentration of the sulphate in plasma falls to about 400th part of its original in 24 hours. Both salts after injection remain in high concentrations in the liver and in the kidney where they can be found for weeks. According to Lourie and Walker (1951), antrycide ‘gives a sharp peak in blood concentration on the height of which survival of the host depends. Trypanosomes are readily killed at levels in the blood lower than those which kill the host.’

Goodwin and Walls (1950) found the phenanthridinium derivative dimidium bromide somewhat more active in mice than antrycide, when tested by subcutaneous or intraperitoneal injection, against 2 strains of *T.congolense*, but they refer to the possibility that the discrepancy was due to their having used strains different from the one employed by Curd and Davey. Further, they found antrycide methylsulphate 3 times as toxic as dimidium bromide when used subcutaneously, but equal in toxicity when given intravenously. Andrianne (1952), working in Belgian Congo, found antrycide salts to be of low toxicity and also effective both for curative and prophylactic purposes.

Roubaud and Bourdie (1950) found antrycide curative for rats and mice in doses of 2·5 mgm. per kilo. against *T.brucei*, *T.congolense* and *T.evansi*. Some relapses, however, occurred in mice even after doses of 5 mgm. per kilo., but only in the case of massive infection, when the flagellates were particularly numerous in the blood at the time of treatment. Roubaud and Bourdie's protocols are given below (the dosage of drug shown was per kilo. body weight):

1. *T.brucei*. A.Mice.—8 infected on 6.XII; all positive on 10.XII. 3 received the sulphate at 2·5 mgm., 3 others at 5 mgm., the remaining 2 being kept as controls. On 12.XII the treated ones were negative for parasites. The controls died by 14.XII. One of those given 2·5 mgm. relapsed 43 hours after treatment.

B. Rats.—7 infected on 14.I; all positive on 17.I. 2 received drug at about 2 mgm. The control died on 18.I. The treated ones were negative by 19.I.

2. *T.congolense*. A. Mice.—7 infected on 29.XI; all positive on 2.XII. 3 received drug at 2·5 mgm. and 3 at 5 mgm. 1 left as control and it died on 5.XII. By 6.XII all treated ones negative. 2 of the mice treated with 5 mgm. relapsed after 16 and 44 days respectively and one of those that received 2·5 mgm. relapsed after 42 days.

B. Rats.— 6 infected on 17.I. 5 treated with 2 mgm. on 22.I on appearance of parasites in blood. 1 kept as control and it died on 24.I. No relapse in any of the treated ones.

8 infected on 9.II. 7 treated on 13.II on appearance of parasites in blood. 1 kept as control and it died on 15.II. No relapse observed in those treated.

3. *T.evansi*. A. Mice.—7 infected on 28.XI. 3 received drug at 2·5 mgm., 3 at 5 mgm. and 1 left as control. This last died on 1.XII. The treated ones negative by 3.XII. One of those treated with 2·5 mgm. relapsed 28 days after treatment and died on the 31st day; another relapsed 3 days after treatment and died on the 37th day.

B. Rats.—7 infected on 13.I. 6 treated on appearance of parasites in blood and 1 kept as control. It is not stated whether the control died. 3 received drug at 2 mgm. and 3 at 5 mgm. The treated ones negative the following day. No relapse.

5 infected on 21.I. 4 received drug at 2 mgm. and 1 kept as control. The treated ones negative and no relapse. Control died.

These workers subjected a goat to the bites of *Glossina calliginea* and as a result the animal showed *T.vivax* and *T.congolense* in the blood on 15.XI and 21.XI respectively. The condition of the animal became very bad and on 1.III it received drug at 5 mgm. It subsequently steadily progressed to complete recovery.

Roubaud and Bourdie tested the prophylactic value of antrycide chloride against *T.equinum* and *T.evansi* when the drug was injected at 5 mgm. per kilo. 8 inoculated with *T.equinum* 12 days after receiving the drug failed to develop the infection. The control died. Their results with *T.evansi* were as follows:

I. 5 rats inoculated with parasites 22 days after receiving drug. None developed infection. Control died.

II. 3 rats inoculated with parasites 37 days after receiving drug. 1 died after 40 days without showing parasites. 1 died after 66 days and the 3rd after 83 days, both of these showing large numbers of parasites in blood. Control died.

III. 3 rats received drug on 2.II and infected on 20.III. All without parasites on 5.V; 1 died on 6.V without showing parasites. 1 showed parasite on 19.V and continued to do so till 16.VI. The third negative. Control died.

IV. 4 mice received drug on 2.II and infected on 11.III. 2 showed parasites on 14.III; 1 died on 22.III and the other 25.III. The 2 others showed numerous parasites on 30.III. Control died.

V. 4 mice received drug on 2.II and infected on 20.III. All showed numerous parasites on 25.III. 2 died shortly afterwards and the other 2 continued to show massive infection till 30.III.

These trials show that protection conferred by antrycide chloride does not exceed 20 days and that, when the animals develop the disease after a protective inoculation, it runs a chronic course, although the parasites lose none of their virulence for healthy animals, which die in 4 or 5 days after receiving an infective inoculation with the parasites. These workers, therefore, stress the need of great care in using the drug prophylactically, for the infection may progress slowly or become 'inapparent' in the treated animals, which, in consequence, become reservoirs of the virus.

Evans (1950) records having obtained, in 1949, striking results in Anglo-Egyptian Sudan from the use of antrycide methylsulphate, by subcutaneous doses of 5 mgm. per kilo., among 250,000 head of cattle, about 25 per cent of which had been found to be infected with *T. vivax* and *T. congolense.* The treatment carried out over a period of several months resulted in infection being almost completely stamped out from the locality.

Discussing the results of experiments on the treatment of cattle with antrycide in Sudan and East Africa, Davey (1950) states that the species of trypanosomes involved in these cases were *T. congolense* and *T. vivax.* The drug was also tested on some camels infected with *T. evansi.* The minimum curative dose in cattle against *T. congolense* is given by him as 2 to 4 mgm. of the chloride and 1 to 2 mgm. of the methylsulphate per kilo., while about 5 mgm. per kilo. of both salts is recommended for use against *T. vinax,* the drugs being injected subcutaneously. The results of prophylactic experiments showed that 5 mgm. per kilo. protected cattle against *T. congolense* and *T. vivax* for 20 weeks. Davey gives an account of a series of prophylactic tests on cattle in fly areas in Uganda and Kenya. This account is summarized in Table XV.

TABLE XV

Results of prophylactic tests on cattle with antrycide against T. congolense *and* T. vivax *infections*

Number of cattle	Drug per kilo	Result
10	2 gm. methylsulphate	3 found infected 11 weeks after
10	1·5 gm. chloride	1 found infected 12 weeks after
12	1·5 gm. methylsulphate and 0·5 gm. chloride	None found infected 12 weeks after
11	1·5 gm. methylsulphate and 1 gm. chloride	None found infected 12 weeks after
11	1·5 gm. methylsulphate and 2 gm. chloride	None found infected 12 weeks after
10	methylsulphate (dose apparently 1·5 gm.)	1 found infected 9 weeks after

Davey refers to laboratory experiments as having indicated that relapse strains show only a small degree of resistance to treatment with antrycide. He states that, while the dose of methylsulphate for use in the field is 5 mgm. per kilo., a dose up to 12 mgm. per kilo. is safe, the former dose having been used with consistent success in the treatment of 200,000 cattle in Sudan. Davey recommends methylsulphate only for curative purposes.

Tobback (1950), Veterinary Counsellor to the Ministry of Belgian Colonies, had a series of extensive experiments carried out with antaycide methylsulphate in the treatment of bovine trypanosomiasis in Belgian Congo. The results of these trials were as follows:

Region A

Series I. All naturally infected with *T. vivax.*

Cow 1. Weight 300 kilo. Emaciated, anaemic, with hypertrophied ganglia.

6.VII.49. Subcutaneous injection of 1·5 gm. methylsulphate in 10 per cent solution.

8.VII.49. Died.

Cow 2. Weight 325 kilo. Condition as above.

6.VII.49. Injected drug as above.

11.VII.49. Blood negative.

18.VII.49. Blood negative. Died.

Cow 3. Weight 425 kilo. Condition good.

6.VII.49. Injected 2 gm. methylsulphate.

25.VII.49. Blood negative.

Series II—2 heifers naturally infected with *T. congolense*, each weighing 250 kilo.

14.VII.49. Subcutaneous injection of 1 gm. methylsulphate.

22 and 26.VII.49. Blood negative.

Region B

Series I. A batch of 28 animals naturally infected with *T. congolense* and *T. vivax* were treated with methylsulphate at 5 mgm. per kilo. in a 10 per cent solution. The animals comprised 12 cows, 4 bull calves, 11 heifers and 1 bull. 7 of the animals had been weakened by the disease, but the others were apparently in good condition The post-treatment observation period was 49 days in 7 animals, 37 days in 10 animals and 24 days in 11 animals. Death occurred in the case of 1 cow with *T. vivax* infection and 1 heifer with *T. congolense* infection; the remaining 26 animals progressed to recovery.

Series II. 11 bovines naturally infected with *T. congolense* treated with methylsulphate, by subcutaneous, intramuscular or intravenous route, at 5 mgm. per kilo., 2·5 mgm. per kilo. and 1·2 mgm. per kilo. respectively, in solutions of 1 or 2 per cent. All effected a recovery except 2, one of which died of theileriasis and the other in the process of intravenous injection.

Series III. 11 bovines naturally infected with *T. vivax* treated as above. All effected recovery except 3; 2 of these 3 died of suspected theileriasis and anaplasmosis and the 3rd of 'en hypothermie; suspicion theileriose'.

Region C

Series I. 9 bovines naturally infected with *T.congolense* and 2 with *T. vivax* treated with methylsulphate at doses varying from 5 to 7 mgm. per kilo. The longest post-treatement observation period was 34 days (the route of injection not stated). Recovery was obtained in all except 1 cow which died, a month after treatment, of an undiagnosed disease.

Series II. 22 bovines naturally infected with *T. vivax* and 10 with *T. congolense* injected by the subcutaneous route, with methylsulphate at a dose of 5 mgm. per kilo. 4 of the treated animals appear to have died without it being possible to perform an autopsy on them, but death in these cases was not attributed to the action of antrycide. Thienpont (1950), who made the observations in this series, states that he followed up the history of the surviving animals for 6 months. He found that one of the *vivax*-infected animals was infected with *T. congolense* 144 days after treatment and 2 *vivax*-infected animals relapsed 52 days and another 144 days after treatment. In a communication published in 1951, Thienpont records having used the methylsulphate subcutaneously in 57 cattle; there were 12 relapses and 7 deaths. In some instances, the injections resulted in shocks.

Wilson (1950) failed to obtain cure, in 2 cattle experimentally infected with *T.congolense*, by the subcutaneous injection of anrycide dimethylsulphate at 0·5 mgm. per kilo. and 1 mgm. per kilo. respectively; both the animals relapsed after the disappearance, for a very brief period, of the parasites from the circulating blood. Both appeared to be cured after re-treatment, each with 3 doses of the drug at 5 mgm. per kilo., at different intervals during a period of 13 days, after they had relapsed. A subsequent experiment showed that the strain was resistant to 2 mgm. of dimidium bromide per kilo. and to 3 mgm. of antrycide per kilo. two out of a batch of 4 non-infected animals proved resistant when they were prophylactically treated with 5 mgm. of methylsulphate per kilo. and then challenged, 2 weeks later, with this strain of *T.congolense*. None resisted the infection when challenged 4 to 7 weeks after prophylactic treatment.

Schoenaers (1950), working in Ruanda, found that, when subcutaneously administered at 5 mgm. per kilo., the methylsulphate cured 13 cases of *T.congolense* infection, the animals remaining free from the parasites for 5 weeks. In 7 animals infected with *T. vivax*, the drug sterilized the circulation in 48 hours, as against 6 to 8 hours in *T.congolense* infection. These observations would appear to be in accord with those of Guyaux (1950, *infra*). A *vivax*-infected bull calf that had relapsed 2 months after treatment was successfully re-treated by Schoenaers with the drug.

Guyaux (1950) records the results of a series of trials carried out by him in Belgian Congo in the treatment of *T. congolense* and *T. vivax* infections in cattle by the subcutaneous injection of the methylsulphate, used at 0·5 gm. per 75 to 100 kilo., 1 gm. per 100 to 200 kilo. and 1·5 gm. per 200 to 350 kilo. From the particulars given by him, it would appear that, while antrycide cleared the circulation of *T.congolense* in 4 to 7 hours, it took 12 to 48 hours to do so in the case of *T.vivax* infection. The injection of the drug was followed by the occurrence of severe local swellings, inappetence and sometimes diarrhoea, while, in one instance, death resulted after an intravenous injection of 1 mgm. of the drug per kilo. The net results of 5 trials were as follows: (1) 55 *T.congolense* cases were cured but about 8 per cent relapsed after 5 months; 15 *T.vivax* cases were cured with about 7 per cent relapses after 1¼ months. (2) 32 animals infected either with *T.congolense* or *T.vivax* were cured, with 3 relapses. (3) Similar results in 22 artificially infected animals. (4) 64 relapsed or reinfected animals or previously treated with dimidium bromide were cured by antrycide and remained free from parasites during an observation period of 84 days. (5) All of a group of 100 graded cattle infected on exposure to tsetse bites were cured after 1 injection, apparently without the occurrence of relapse in any of the animals; in those placed in fly areas, re-infection occurred in 30-120 days. According to this worker, antrycide is the most effective drug for use in bovine trypanosomiasis. Jezierski (1951) likewise refers to the drug as being highly effective in the treatment of trypanosomiasis of cattle, sheep and goats. Ferreira and Tendeiro (1950), working in Portuguese Guinea, used with almost uniform success a single dose of 1 mgm. of the methylsulphate per kilo. in the treatment of 13 cattle, 7 horses and 1 donkey. Of these 21 animals, 18 were infected with *T.congolense* and 1 each with *T.vivax*, *T.brucei* and an undetermined species of trypanosome. None of the animals relapsed during a post-treatment observation period of 6 to 9 months, except in the case of a *congolense*-infected cow which died 34 days after the injection, although the results of blood smear examination in this case were negative. Animals artificially infected with *T.congolense* were likewise successfully treated. A dog after receiving an injection of antrycide (apparently the chloride) 'lived for 6 months after being inoculated with 5 c.c. and re-inoculated (after 16 days) with 10 c.c. of the blood of a dog with *T.congolense*'.

Perhaps the most valuable testimony to the efficiacy of antrycide is provided by the minutes of the Conference of the International Scientific Committee of Trypanosomiasis Research held at Antwerp in June 1950 (Abstr. in *Trop. Dis. Bull.*, **48**, 338; 1951). In this conference antrycide was declared to be the most effective drug for therapeutic purposes, although its prophylactic value still remained to be finally determined. The conference referred to its high cost as its drawback. A similar testimony—this is in regard to the prophylactic value of the drug—is contained in an undated report (published from Leopoldville, Belgian Congo) of the proceedings of the Third Session of the International Scientific Committee held in June 1951. The drug used is stated to be methylsulphate, the locality of experimentation being Mozambique. The same session would appear to have appointed a sub-committee of veterinary officers to report upon the position in regard to the use of antrycide and dimidium bromide in the treatment of animal trypanosomiasis. The main findings, of this sub-committee were as follows. In the recommended dose of 5 mgm. per kilo. for antrycide methylsulphate and 1 mgm. per kilo. for dimidium bromide, the cost of the former comes to about 3 times that of the bromide, which latter is, therefore, the drug of choice for use in the case of animals of small value. Furthermore, the bromide is more easily prepared and better tolerated. On the other hand, delayed poisoning has been reported after dimidium bromide. Antrycide is effective against *T.evansi* in camels and partially so against *T.brucei* and *T.simiae*. None of these 3 species of trypanosomes is amenable to treatment with dimidium bromide. Prophylactically the pro-salt (containing the methylsulphate and chloride salts in the proportion of 1·5:2) is distinctly superior to dimidium bromide, for the former confers protection for at least 2 months after a single injection, as against 1 month's protection conferred by dimidium bromide. The sub-committee stresses the importance of the fact that, in order to obviate the possibility of the production of antrycide-fast strains, the drug should not be prophylactically injected at longer than 2 months' interval.

Pellegrini and Bonelli (1951*a*) in Somaliland tested the efficiency of antrycide in the treatment and prevention of trypanosomiasis both in the laboratory and in the field. It would appear that they used the drug on several species of animals, including cattle, horses, mules, camels, sheep, dogs and cats, the species of trypanosomes involved being *T.congolense*, *T.vivax*, *T.brucei*, *T.evansi* and *T. simiæ*. The pro-salt is stated to have conferred protection for a maximum of 6 months. What would appear to be an account of these authors' same observations, was published in another paper (Pellegrini and Bonelli, 1951*b*), which the compiler of this monograph has been able to consult in original. They used the methylsulphate in the following doses for larger animals, the drug being used in all cases in a 10 per cent aqueous solution: Animals weighing up to 150 kilo. at 5 mgm. per kilo.; those weighing from 150 to 200 kilo. at 1 gm. per dose; those weighing from 200 to 350 kilo. at 1·5 gm. per dose; and those weighing from 350 kilo. at 2 gm. per dose. Dogs, cats, sheep and goats were injected at 5 mgm. per kilo. in a 1 per cent solution. In all cases, the drug was administered by the subcutaneous route. Antrycide did not provoke any general reaction in the majority of bovines, except for frequent ptyalism, accelerated respiration and lachrymation, all of which, however, soon disappeared. There were symptoms of uneasiness ('etat d'agitation)' immediately following the administration of the drug. this being particularly noticeable in the horse. The intravenous injection of the drug may, according to them, result in grave symptoms terminating in death. The local inflammatory reaction was negligible. The results of the therapeutic treatments were as follows:

T. congolense

Bovines. 20 adults and 8 calves. All the adults recovered but 4 of the calves died unexpectedly while grazing; the cause of death could not be determined. Immediately after treatment, the animals were kept in a fly area and trypanosomes re-appeared in 8 of them in 69 to 119 days after treatment—5 showed *T.congolense*, 2 *T.vivax* and 1 an unidentified species of trypanosome. Of these 8 animals, some were subjected to a second treatment with the methylsulphate and the others with the pro-salt . Cure was obtained in all cases.

Camels. 2 infected animals treated with a single injection and both cured.

Dogs. 4 animals treated. 1 died some hours after treatment; the others were cured.

Sheep. 8 animals treated. 1 died 4 hours and a second 36 hours after treatment; the others cured.

Horses. A single animal, that had developed extensive ulceration at the site of injection after having been prophylactically treated with the pro-salt, was infected with *T.congolense* 26 days after being placed in a fly area. It was successfully treated with a single injection of the methylsulphate.

T.vivax

Bovines. 10 adults treated and all sterilized in 24 hours after drug injection. In 1, the parasites re-appeared for 2 days in the blood 12 days after treatment ; 2 died, 13 and 16 days respectively, and a third 4 months, after treatment. The cause of death in these cases was undetermined.

T.evansi

Camels. 4 successfully treated. 1 died of purulent peritonitits.

Dogs. 4 experimentally infected. All cured, but one died of filariasis. One of the dogs was succesfully re-infected with a different strain of *T.evansi* but was again cured with antrycide.

Cats. 6 experimentally infected by the oral and rectal routes. In 1 the drug failed to sterilize; in the others the parasites returned in 10, 11, 14, 42 and 66 days after treatment; 1 died of trypanosomiasis 20 days after treatment. In the others the infection assumed a chronic form and was not affected by repeated treatment.

T.brucei

Bovines. 3 animals after being successfully treated were kept in a fly zone. 1 became infected after 119 days. The others remained in good condition.

Solipedes. 1 experimentally infected horse and 5 naturally infected donkeys treated. All cured but the horse died 10 days after treatment and 1 donkey died 2 months later. Both were old and ill-nourished.

Camels. 1 animal cured. Contracted *T.congolense* infection and died 68 days after being placed in a fly area, in spite of the fact that it had developed a benign form of the infection that had been controlled by antrycide.

Sheep. A single animal successfully treated.

Dogs. 3 natural cases and all cured. 2 experimentally infected dogs also treated. Of these, 1 relapsed about 13 days after treatment; when re-treated with a double dose (10 mgm. per kilo.), it died after showing symptoms of intoxication. The other, which relapsed after about 21 days, was given an intravenous injection of 0·5 gm. suramin and was only temporarily sterilized after this treatment; it showed periodic re-appearance of the parasites in its blood, although from the clinical point of view it was regarded as cured.

T.simiae

Camels. 5 naturally infected animals were successfully sterilized by the drug, but 4 of them died in quick succession 10 days after treatment. The 5th recovered.

For prophylactic purposes, Pellegrini and Bonelli used the pro-salt and found that it protected for a minimum of 2 and a maximum of 6 months.

Suter (1951) studied the protective action of pro-salt in 20 oxen, the drug being used at about 5 mgm. per kilo.; 20 other oxen were kept as controls. One of the treated animals showed trypanosomes in blood after 126 days' exposure to fly bites, while after 7 months' exposure 15 of the treated and 46·8 per cent of the untreated showed the parasites. According to Suter, the duration of protection conferred by the pro-salt on cattle against *T.vivax* is about 4 months.

The value of the pro-salt as a prophylactic agent was well brought out in the course of 2 experiments carried out by Mornet, Lalanne and Cissoko (1952) on 2 batches of bovines during their journey through a tsetse area. The first experiment was preformed on a group of 100 zebus; the distance covered by them was about 1000 kilometers over a period of 51 days. In the second experiment, 120 animals travelled over a distance of 500 kilometers over a period of 31 days. In the first, the animals were divided into 5 equal groups of 20 each, the first 4 of these being dosed with trypadine (dimidium chloride), lomidine, potassium antimonyl tartrate and antrycide respectively; the fifth group was left as controls. In the second experiment, the 120 animals were divided into 6 groups of 20 each, the first 4 of these being treated as in the preceding experiment, the fifth treated with suramin and the sixth left as controls. The main points that emerged from these experiments were : (1) the intravenous injection of drugs—such as in the case of dimidium chloride and tartar emetic—was hardly practicable where a large number of animals were involved and in this respect antrycide proved most suitable; (2) the percentage of infection was insignificant at the time of commencement of the journey and became very high on arrival at destination; (3) antrycide is the only dependable drug for absolute protection over a period of 1 month—a statement based on the result of the second experiment, the result of the first being inconclusive; (4) the preceding statement is supported by the fact that, while 70 per cent of the control animals were found infected on arrival, all the antrycide-treated animals remained free from infection; (5) dimidium chloride was only slightly inferior; (6) suramin, lomidine and tartar emetic were ineffective, the last causing death.

Unsworth and Birkett (1952) used the pro-salt in protecting a herd of cattle during their journey through a tsetse belt in Nigeria. The pro-salt was used at 15 ml. per kilo., up to a maximum of 20 ml. per animal; 10 animals were kept as controls. These workers thus summarize the results of their experiments: 'A single inoculation of antrycide pro-salt afforded complete protection against trypanosomiasis for 59 days to cattle travelling on the hoof through areas heavily infested with *G.morsitans*, together with some riverine tsetse (*G.palpalis* and *G.tachinodies*). All untreated cattle which travelled with the treated animals became infected and 2 died of trypanosomiasis by the 45th day of the trek'. The untreated animals lost an average of 13 kilo. and the treated ones gained an average of 11 kilo. in body weight. One of these workers (Unsworth, 1952) also attempted to control an outbreak of *T.simiae* in pigs with the methylsulphate used at 3 to 5 mgm. per kilo. The piggery contained a herd of 23 sows and 8 baconers and all died in quick succession in spite of the treatment. Antimosan was also unavailing in controlling the outbreak.

In India, the value of antyrcide salts was tested by Ray *et al.* (1953) on 116 cattle and 21 horses naturally or artificially infected with *T.evansi* and this constitutes the only record of the use of antrycide in this country. They employed the methylsulphate and pro-salt in doses from 3 mgm. to 14·8 mgm. per kilo.; there were no local reactions. Two groups of 33 infected cattle each were treated with the methylsulphate and pro-salt respectively, while 33 others were kept as controls. When subjected to complement fixation test 3 months later, all treated animals proved to be free from infection, while 48 per cent of the control animals were positive and 12 per cent doubtful. The drugs were administered in single subcutaneous injections. According to these workers, 'the methylsulphate gives a residual protection' and the pro-salt gives a solid protection for some months. They worked out the cost of antrycide treatment for an animal weighing 200 to 300 kilo. as approximately 25 per cent and 50 per cent of the cost of a course of suramin treatment, for methylsulphate and pro-salt respectively.

Antrycide has also proved effective in the treatment of dogs naturally infected with *T. congolense.* Alley (1951) successfully treated 5 such infected pariah dogs with the methylsulphate at 0·5 c.c. of a 10 per cent solution per 15 lb. body weight, 2 of these dogs being 'emaciated and unable to stand—1 was suffering from dysentery—and it was considered that any treatment would be of little use'.

In view of their remarkable therapeutic and prophylactic properties, antrycide salts would have ranked as the ideal agents for use in animal trypanosomiasis if they had shown themselves to be free from the drawback of producing drug-fast strains. Unfortunately this is not the case. Wilson (1949) in Uganda found that a single dose of 3 to 5 mgm. of the methylsulphate cured pigs infected with *T. simiae.* One pig, however, relapsed after 2 mgm. of the chloride per kilo. and resisted immediate treatment with a dose of 5 mgm. of the methylsulphate per kilo. He observes: 'the relapsed strain was considered, therefore, to have developed drug resistance in this short time'. In another experiment, 10 cattle injected with 2 gm. each of antrycide methylsulphate were exposed to tsetse bites. From the only animal that survived, 6 months later a strain of *T. congolense* was collected and subinoculated into a healthy calf which, on developing the disease, resisted treatment with 5 mgm. of methysulphate per kilo. Six mice infected with the same strain were treated with the methylsulphate at 10 mgm. per kilo. and 2 of them relapsed 52 days later. Some were re-infected a few months after being cured with antrycide, but when re-treated with this drug 'developed cryptic but nevertheless fatal infections'. Wilson obtained experimental evidence pointing to the conclusion that antrycide resistance also involved resistance to dimidium bromide.

In discussing the relative merits of antrycide and dimidium bromide, Fiennes (1951) refers to his own experience as showing that the latter was more effective against *T. congolense* though not against *T. vivax,* a dose of 1 mgm. per kilo. having cured all of a group of 7 cattle and a dose of 0·5 mgm. per kilo. 20 out of a batch of 33 cattle infected with *T. congolense*. The strain of *T. congolense* was the same as that used by Davey in his antrycide work. He, however, concedes that 'antryside, in many ways, is the best trypanocide yet produced.' He further points out that the recommended dose of antrycide is 5 mgm. per kilo. while that of dimidium -bromide 1·2 to 1·5 mgm. per kilo. and this makes dimidium chahper for use in the case of large numbers of native stock. Antrycide, however, possesses the great advantage of lending itself for successful use against all species of trypanosomes affecting stock in Africa. Fiennes stresses the complex nature of the problem of dealing, under field conditions, with chronic or 'secondary' trypanosomiasis, which may often prove resistant to treatment, although the parasites concerned are not drug-fast. In such cases, the condition may progress to a fatal issue without the parasites being present in the peripheral circulation, although they may eventually be found in the cardiac muscles. According to him, 'pathological symptoms and lesions may occur in the absence of blood infection'. In any case, the suitability of a drug for general use does not depend on its efficacy alone. As pointed out in a note which appeared in the *Bur. interafr. Tsetse tryp.*, 1951 (cited in *Vet. Bull., Weybridge*, **22**, 458: 1952), the question of the superiority of this drug over its near rival dimidium bromide will have to be determined on the combined basis of cost, dosage, mode of administration, reaction, toxicity and effectiveness.

In a thoughtful article, Fiennes (1952) discusses the question of antrycide prophylaxis vis-à-vis the future trypanosomiasis of cattle in Africa. In reviewing the results of work carried out by him during a period of 3 years, the author first refers to the laboratory experiments of Davey (1950) as having shown that antrycide chloride, when administered subcutaneously at a dose of 10 c.c. of a 20 per cent suspension, conferred protection against *T. congolense* for 5 months and against *T. vivax* for 3 months or more. When, encouraged by these laboratory results, Davey placed groups of treated cattle in fly areas, many died within the space of a few months, often without showing parasites in the circulating blood, although all clinical and post-mortem evidence pointed to the conclusion that the animals had died of a 'cryptic' form of trypanosomiasis. Those that survived eventually showed parasites in the blood and resisted re-treatment either with antrycide or with dimidium. Two further groups of cattle prophylactically treated with antrycide, when exposed to tsetse bites, soon contracted *T. congolense* and *T. vivax* infections, the majority dying within 3 months. Trypanosomes collected from them resisted the action of antrycide. It was possible to collect drug-fast trypanosomes from the area during a period of 7 months by 'introducing untreated cattle into the area after the removal of all treated cattle'. These observations point to the danger of unrecognized drug-fast trypanosomes being spread by flies from animals apparently, but not actually, cured by antrycide. The results of an experiment carried out by Fiennes showed that natural resistance to trypanosomiasis could be induced in calves reared from dams protected by antrycide in a tsetse area. Davey (1951) had admitted that, while the chloride was less toxic than the sulphate, it was more liable to cause the development of resistant strains.

CHAPTER XVI

Miscellaneous Drugs

In THIS section, it is proposed to consider a number of drugs that have been more or less empirically used in the treatment of animal trypanosomiasis, but they might, nevertheless, provide useful indications as to the lines on which future work in this field might lead to the discovery of more effective remedies. A few of these have been incidentally mentioned in the Introduction.

INORGANIC SALTS

Sazerac and Larthe (1935) mention Perla (1934) as having shown that the oral administration of copper sulphate for 12 days has a protective action against *T. equiperdum* infection in rats. They themselves used an oily suspension of copper protoxide in the treatment of rats infected with *T. brucei*; they obtained only a temporary sterilization when the drug was used at 20 mgm. per 100 gm.

The chloride of rhodium—a rare metal of the platinum group—was tested by Lagodsky (1938) for its trypanocidal action in mice when it was injected subcutaneously or intravenously, but the drug proved of no value.

ARSENICALS

Castelli (1912) tested the efficacy of sodium cacodylate and arrhenal for nagana with disappointing results.

In view of the reported successful use by Lieurade (1934) of intravenous injections of a combination of urotropine and tryparsamide, Van den Branden and Pottier (1934) tested it against *T. congolense* in rats; the combination appeared to possess no advantage over tryparsamide alone.

ALKALOIDS

Schuscha (1917) unsuccessfully used the sublethal dose of 0·001 gm. of emetine hydrochloride against *T. equiperdum* in mice.

Opium alkaloids were tested by Macht and Weiner (1918) on *T. brucei* but they were found ineffective. Pavaverin—constituting about 1 per cent of opium—would appear to have been mainly the alkaloid used by them. It exerted an inhibitory action on the trypanosomes *in vitro* but it had no curative effect on rats infected with *T. brucei*.

Quinine and its various preparations were, in the early days of chemotherapy, subjected to frequent tests in the treatment of trypanosomiasis and were not found altogether valueless, although they did not lend themselves for use as actual chemotherapeutic-remedies. Morgenroth and Halberstaedter (1910*b*) tested quinine against nagana mice and found that it was not trypanocidal but that it checked the multiplication of the parasites. According to them (1911*a*), hydroquinine is much superior to quinine. They (1911*b*) further found that ethyl hydrocuprein, which is the next highest of the homologues of hydroquinine (quinine itself being methylcuprein) was still superior. These results led them to suggest trials with the higher homologues of the series and their combination with other trypanocidal drugs in the treatment of animal trypanosomiasis.

DYES

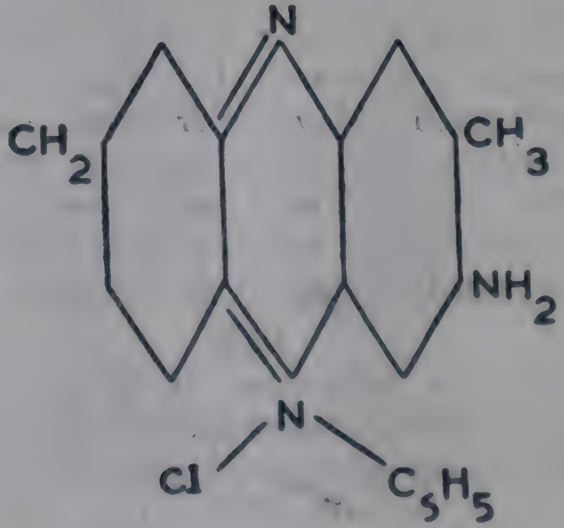
Dyes in one form or another have now and again received considerable attention as holding out a possibility of proving lethal for pathogenic trypanosomes in view of their predilection for particular tissues of these parasites. Foy (1909) mentions Brand as having successfully used a mixture of a solution of methylene blue and mercuric chloride intravenously in the treatment of 3 cases of equine trypanosomiasis. In practice, it would seem hardly advisable on principle to use strong inorganic salts intravenously as being liable to prove fatal for animals.

Brieger and Krause (1912*a*) showed that drugs of the safranin group (diamino derivatives of azonium bases) possessed trypanocidal properties and they also made the important observation that the action of these drugs depended on their side chains, interference with which substantially altered their efficacy. According to them, the failure on the part of previous workers to obtain recovery by the use of various drugs was possibly due to the fact that they were rapidly disintegrated in the blood , so that they had only time to act on parasites present in the circulation and were ineffective against those lodged in the deeper tissues. They found that drugs belonging to the safranin and eurhodine groups were stable.

Laveran and Roudsky (1912) made the interesting observation that acridine and oxazine had a special affinity for the centrosome of *T. lewisi* but not for the centrosomes of pathogenic trypanosomes and they offer what would appear to be a plausible explanation for this phenomenon, but it would be outside the purview of the present monograph to consider their views on this subject. As for the therapeutic value of acridine, Salmon (1913) found it effective against *T.gambiense* in mice but its action was less energetic than that of arsenicals. Its therapeutic index was 5 and recovery was obtained in 3 out of a group of 4 infected mice treated with 0·04 to 0·25 mgm. of the drug. Salmon found that certain dyes of the acridine or oxazine series exerted a tripanocida action, attacking the blepharoplast and thereby eventually destroying the parasites.

As dyes often produce pain when they are given by injection, Brieger and Krause (1912*b*) discuss the possibility of using only non-poisonous dyes that could be given by the mouth in the treatment of trypanosomiasis. They 'sought for bodies which, in consequence of a favourable combination of nitrogen-containing groups with 3 and 5-valent nitrogen, could be taken by the mouth without poisonous action and permanently destroy the trypanosomes'. According to them, substances of the safranin group might fulfil such a condition. They base this view on experimental observations made by them on artificial *T. brucei* infection.

In one of the papers published by Brieger and Krause (1912*a*), they elaborated their observations on the effects of dyes of the safranin group on *T.brucei* infection in rats and guinea-pigs. They found that a body of the following general formula was the most effective when given by the mouth:



The animals were fed for periods from 4 to 6 weeks, the treatment being given simultaneously with the infection or up to 6 days after the infective inoculation. The drugs were found safe to use in the case of human beings in doses of 0·25 to 1 gm. daily up to a total of 30 gm. On the other hand, Ritz (1913) records having failed to use safranin successfully in the treatment of *T. brucei* infection in mice, rats and guinea-pigs, while Lurz (1913) also mentions the drug to be valueless. Brieger and Krause (1914), in a rejoinder, state that all of a batch of 44 guinea-pigs treated with trypasafrol (the same as safranin) were alive on the 90th day after treatment, while the controls died. In a paper published by them in 1915, Nuttall and Hindle state that they too found the drug altogether ineffective when tested against *T. brucei* infection in guinea-pigs.

Trypaflavine or acriflavine—also a derivative of acridine—was tested by Russo (1914*b*) on *T. brucei* in rats and was found by him to be toxic; when used in combination with basic optochin, it exerted an active action. Berger (1926) mentions a dye called 'peracrina 303' and stated to be a trypaflavine-albumen combination containing 10 per cent trypaflavine. On being tested on mice infected with nagana, it proved to be inferior to trypaflavine.

The 2 dyes trypanblue and tryparosan (dichloroparafuchsin) were experimented upon by Rodhain and Van den Branden (1916) for their efficacy in *T. congolense* and *T. cazalboui* infections in laboratory animals. Only tryparosan proved effective when administered by the mouth at 0·5 gm. per kilo.

Browning *et al.* (1935) experimented with a series of selenazole analogues of styryl quinoline to test their trypanocidal action on *T. brucei* in mice. They proved to be almost non-effective. Browning and Gulbransen (1935) made the interesting finding that spontaneous cure of untreated relapses in mice infected with *T. brucei* took place if these relapses followed treatment with styryl quinoline compounds and acridine derivatives. Possibly, such recovery is—at any rate partly—due to the slow excretion of the drug, thereby continually exerting some trypanocidal action on the parasites. Parkin (1931*c*, 1935*d*) records having successfully used styryl quinoline in the treatment of *T. brucei* infection in equines, at 0·004, gm. per kilo. injected intravenously twice at a weekly interval. Better results were obtained when 5 half-doses were employed at intervals of 1 week or 24 hours.

The trypanocidal actions of azo dyes chlorazol fast-pink, chlorazol fast-blue and what has been designated as 'S.D.2' were compared by Huggett and Suffolk (1936) with the action of suramin on *T. equiperdum* in mice. Chlorazol fast-pink was nearly as effective as suramin but its therapeutic index was much lower. All the three azo dyes, when administered during the incubation period, delayed the appearance of the trypanosomes.

The possibility of using pyrrole dyes as trypanocides was examined by Fischl (1935). Four of these were tested by him, namely, prodigiosin, pyrrole blue, pyrrole red and phthalocyanin; only the first showed some trypanocidal activity. Mansjoer (1948) tested the efficacy of large doses of trypaflavine for surra and found the drug valueless.

QUINOLINE AND GUANIDINE DERIVATIVES

Synthalin (dimethylene diguanidine hydrochloride) has been tested by a number of workers for its trypanocidal action. Lourie and Yorke (1937) found that it was strongly trypanocidal *in vitro* even at a concentration of 1 : 262,000,000 and that it was also effective against trypanosomes resistant to atoxyl and suramin. These workers also obtained evidence that the drug directly acted upon the trypanosomes and that its action was not in the nature of an addition to the production of hypoglycaemia. Browning (1938) also came to a similar conclusion with regard to the mechanism of the action of synthalin, from his studies upon the effect of this drug on *T. congolense* in mice. The so called 'anticoman' was tested by Pop and Budac (1938) against nagana and dourine in rats. The drug is stated to be a mixture of guanidine preparation of synthalin bitartrate, pancreas powder, sodium phosphate, tannic acid and bismuth subnitrate. The mixture is, according to these workers, less toxic than synthalin alone but it proved to be of low therapeutic value. Anticoman acts directly on the parasites and not by the production of hypoglycaemia.

A notable advance was registered in the field of chemotherapy when Lourie *et al.* (1951), in the course of their studies on the chemotherapeutic values of preparations of salts of quinoline, cinnoline and quinazolene, discovered a guanidine compound with the following formula: N^1: N^3bis (4-amino-cinnolyl-6)—guanidine methiodide. Both in respect of toxicity and trypanocidal power, the drug is claimed by them to be comparable to antrycide. It, however, does not appear to have been tested clinically. Van den Branden (1938*c*) tested the sulphate of ortho-oxyquinoline (chinosol-sunoxol) on white rats infected with *T. brucei*; it proved ineffective. De Borchgrave (1941), however, found that chinosol, while it had no remedial value, made *T. gambiense* in guinea-pigs uninfective when the drug was added in concentration of 1 : 1,000, five minutes before inoculation. The blood could be made uninfective when, in place of chinosol, use was made of plasmochin, yatren, quinine hydrochloride, argoflavine, atebrin, astreptine (sulphanilamide), nipagin or carbolic acid. These drugs may, therefore, be used in making trypanosomes uninfective in blood for rinderpest inoculation. The drug astreptine was tested by Van den Branden (1938*d*) on *T. brucei* in rats, but it was found ineffective.

Reference may be made here to certain drugs which can be only grouped together in the absence of any significant chemical or other relationship amongst themselves that might provide a basis for their classification into categories.

Lamballe (1913) claims to have used successfully pancreatic enzymes in the treatment of relapsed cases of surra in mules. These enzymes were trypsin and amylopsin administered twice in 3,750 units and 1,000 units respectively, at 1-day interval. The treatment of surra was also attempted by Fernandez (1926) with formalin. Using a dilution of ½ to 1 ounce of formalin per pint of water, he found that cure was obtained in camels, horses and cattle when 20 c.c. of the diluted drug was injected intravenously on 13 alternate days and then at 3-day intervals, the entire course occupying a period of 1½ months. For dogs, he recommends a dose of 1 to 3 c.c. of a dilution of ½ to ¾ drachms in 5 ounces of water.

Of other empirical methods for the treatment of trypanosomiasis, mention may be made of the use by Johnston and Macfie (1913) of tincture of iodine, bromine and osmic acid in the treatment of *T. vivax* infection in sheep and against *T. lewisi* in rats, but these chemicals failed to answer to the requirements of chemotherapeutic drugs, for they proved to be both parasitotropic and organotropic simultaneously.

As one would expect, sulphanilamides, on account of their well-known specific action on certain human diseases, have received some attention as to the possibility of their serving as remedies for trypanosome infections. They were, however, found to be valueless by Kolmer and Rule (1939) against *T. equiperdum* and by Van den Branden (1938*d*) against *T. brucei* in rats, as mentioned earlier. Urso (1941) tested sulphathiazol orally and intramuscularly on *T. equiperdum* in guinea-pigs; it proved ineffective.

Rosenthal and Bauer (1941) record having found 3-nitrobenzoic acid and its derivatives—notably, 3-nitrobenzoate of sodium—somewhat effective against *T. equiperdum*, although relapses were common.

e wide range of inquiry made into the problem of finding out effec
osomiasis, mention may be made of a series of experiments carrie
(1944), to test the possibility of using sodium penicillin as a curativ
ion. The drug proved valueless.

dies on the trypanocidal action of drugs, it is important that the
for their remedial effect may in some instances be due not to th
the impurities contained in them. Thus, Keneford *et al.* (1948) men
ives—4 : 6 diamino-1-methylcinnolinium iodide and an analogue–
against *T. congolense* infection in mice but the impure product
were as effective as phenanthridinium compound '897', but 'the try
considerably according to the conditions used for reduction'. Th
by Lourie *et al.* (1951, *ante*) was believed by them to represent th
t of the impurities mentioned by Keneford *et al.* and they desig

made here of petroleum tested by Van Saceghem (1921*b*) and foun
anosomiasis when it was administered *per os*. The same worker (192
eless as a remedy against experimental *T. congolense.*

roprietary preparations the composition of which remains undis
e made of an Italian preparation called 'Albert 102'. Miessner an
t it conferred protection on mice and guinea-pigs against *T. bruc*
ctions for 3 days, while a drop of 1 to 2 per cent solution of this dru
m motionless in a suspension. Another Italian proprietary drug
stated by Coceani (1942) to be curative for *T. sudanense* infection i
) mention a drug designated by them as $C_{10}H_{21}$ O_3N_5H CL, whic
neously at 0·15 gm. per kilo., cured 7 out of a batch of 8 mice infec
A dose of 0·075 gm. per kilo. cured 6 out of 8 mice.

at even cortisone, one of the most recent and valuable introductio
me in for its share of attention. Von Brand *et al.* (1951) tested th
g gluconeogenesis by its use in trypanosome infections but the resu

1937) made use of snake poisons (from *Bitis arietans* and *Na*
tment of infected mice. The administration of these poisons cause
parasites. The dosage of poison in the case of *Naia tripudians* wa
g. to 0·0000 25g. A weak dose of the poison is stated by him

They mention having kept an infected human subject intermittently in a cold chamber; the patient reported an improvement in his general condition but the disease itself remained unaffected. Temperature appears, even under natural conditions, to have some relationship with the course of trypanosomiasis, for Raven (1911*b*) mentions the character of the weather as having been responsible for producing a set-back in some of his sleeping sickness patients.

T. cruzi, the causal agent of Chagas disease, represents a class by itself. The remedial measures adopted by different workers against this trypanosoma being of little significance in relation to the question of dealing with other pathogenic trypanosomes, they are not considered in this monograph. The observations recorded by Lwoff *et al.* (1944) on the treatment of this disease are, however, deserving of mention as supporting the view that chemotherapeutic drugs may act both *in vitro* and *in vivo* or only *in vivo*. These workers tested the activities of the following drugs on *T. cruzi*: (1) derivatives of ethylene diamine, (2) derivatives of naphthalene and (3) a compound containing arsenious acid. The first 2 were found to belong to the first category and the third to the second category.

CHAPTER XVII

Serum Therapy in Trypanosomiasis

ALTHOUGH serum therapy in trypanosomiasis might be appropriately considered in relation to the general question of immunity in this disease, a consideration of this subject raises certain fundamental issues which would be best dealt with in a separate chapter, as is being done here. The larger question of immunity will form the subject of the chapter that follows.

I. NON-SPECIFIC SERUM

The trypanocidal properties of serum of normal animals have been a favourite subject of study among some of the early workers in the field of trypanosomiasis. A useful review of the subject of the trypanocidal action of human serum has been published by Culbertson (1935).

As early as 1906, Minchin, Gray and Tulloch showed that sheep serum was effective against *T. grayi*. Thiroux (1909*b*) found that both this and human serum acted on *T. duttoni*.

In an editorial note in *Sleeping Sickness Bureau Bull.* (1,255; 1909), Laveran and Mesnil are mentioned as having demonstrated that human serum had a trypanocidal action on the parasites of nagana and that, when injected into infected rats and mice, it influenced the course of the disease, with temporary sterilization of the blood. Mention may also be made here of the observations by Mesnil and Ringenbach (1912) and others on the trypanocidal action of the serum of primates on human trypanosomes. It is of interest that Laveran and Nattan-Larrier (1912) showed that *T. rhodesiense*, although it may become resistant to human serum, easily loses this property.

Mesnil and Leboeuf (1910) mention Laveran as having shown that the srum of baboons caused a temporary sterilization in mice infected with *T. gambiense*, *T. evansi*, *T. brucei* and *T. equinum*, but that it was less active than human serum. Mesnil and Leboeuf themselves found the serum of the baboon *Cynocephali* 10 to 25 times more active than human serum. The same authors (1912), working in collaboration with Ringenbach found that trypanosomes which were susceptible to the action of the serum of baboons, were also susceptible to the action of the serum of man and mangabey (an African genus of monkey), but not vice versa, showing thereby that the protective substances in the sera of different classes of primates are not identical. Mesnil and Blanchard (1916) found that 2 strains of *T. rhodesiense* were acted upon by normal human serum while a strain of *T. gambiense* was resistant.

In one of a series of papers published by him on the subject of the curative mechanism of human serum, Rosenthal (1929) gives possible reasons why it is inactive *in vitro* but trypanocidal *in vivo*. He evidently considers that the intravital process whereby the serum becomes trypanocidal is analogous to that of the conversion of pentavalent into trivalent arsenicals, the reticulo-endothelial system having a major share in the process. Rosenthal designates the initial, that is, non-trypanocidal, substance in the serum as trypanocidogenous and the form which it assumes on injection as trypanocidal. Yorke *et al.* (1930) record having failed to confirm the findings of Rosenthal, for they found that human serum *in vitro* was trypanocidal against *T. equiperdum*, *T. rhodesiense* and *T. congolense*, but was ineffective against *T. gambiense* and these facts, they argue, explain why human beings are susceptible to this species but immune to trypanosomes pathogenic to stock. According to these workers, this fact affords 'the strongest grounds for believing that man's immunity to infection with the pathogenic trypanosomes of stock, and his relative susceptibility to *T. gambiense*, are bound up with the property of serum'.

Of the other workers who have studied the protective properties of non-specific serum against trypanosomiasis, mention may be made of Moulis (1938), who claims to have obtained cures in a horse and in a cow by the use of monkey serum when novarsenobenzol had proved ineffective. The serum was obtained from a species of *Cynocephalus* and was given intravenously in doses of 8 to 10 c.c. Lloverol (1939) tested the value of normal serum of the baboon, *Papio papio*, in the treatment of experimental *T. congolense* infection in sheep. The serum was used intravenously in doses of 15 to 20 c.c. Out of a total of 5 sheep, 2 recovered after 2 or more injections 2 died after the parasites had been absent from the circulation and 1 continued to show the parasites in spite of serum injection. All the 3 controls died.

In discussing the curative action of normal human serum on nagana and dourine in mice, Adams (1928) advances the view that it is not an 'irritant body' action ('behrut nicht auf einer reinen Raizkörper-Wirkung'), for the injection of bile, urea, iodine, acids, etc. does not produce any effect on the trypanosomes, although these are known to contribute to the anti-typhoid action of serum. According to him, normal human serum possesses specific trypanocidal properties, which are capable of being improved upon by superimposed syphilitic infection. In his experience, the sterilization produced by the serum was of a transient nature, relapses having been found to occur about 7 days after injection of the serum. He further records having found the serum of sheep and lower primates ineffective, but this is contrary to the observations recorded by several other workers. (e.g. Minchin *et al.*, 1906; Thiroux, 1909; Mesnil *et al.*, 1912).

II. Serum after Infection or Treatment

Mesnil and Brimont (1909), in a lengthy paper, discussed the protective properties of sera of animals infected with trypanosomiasis. In the course of their studies, they came across resistant races in these sera. The species of trypanosomes used by them were *T. equiperdum*, *T. equinum* and *T. brucei*. The main findings that emerged from these studies were : (1) the serum of animals that have developed a subacute or chronic form of trypanosomiasis acquires protective properties very quickly and such serum is, within certain limits, effective only against homologous strains of trypanosomes—properties which, according to these workers, may be utilized for differentiating closely allied forms of trypanosomes; the serum loses much of its protective power after recovery of the animals. (2) Trypanosomes from such infected animals proved to be non-infective for mice and this shows that the parasites had some of the immune bodies fixed in their tissues. (3) The serum was ineffective *in vitro*, but when the serum, together with some actively motile parasites, was injected intraperitoneally into mice, they were rapidly destroyed as a result of phagocytosis. The parasites in the blood of donors of immune serum were themselves refractory to the action of the serum and they retained their resistance in spite of being passaged a number of times. The donors being infected with chronic trypanosomiasis, the occurrence of resistant strains in their blood was only what was to be expected.

Jacoby (1909 *a*, *b*) noticed the occurrence of the sterilizing action of human serum in mice infected with nagana, but there was a progressive recurrence of relapses and complete recovery seldom occurred. Like Mesnil and Brimont (*ante*), Jacoby, obtained a resistant strain, which, although it retained its resistance for 12 generations, gradually lost it through later passages. He records failure to develop a strain that retained its resistance through 100 generations. According to him, the nature of resistance developed by such refractory strains is very different from that by drug-resistant strains. Heckenroth and Blanchard (1913) and Mesnil (1913) also published some observations on the protective properties of serum of human beings affected with trypanosomiasis.

The trypanolytic action of serum of infected animals on homologous or closely allied strains was demonstrated by Leger and Ringenbach (1912) in the case of surra, nagana, mal de caderas and sleeping sickness. The method of testing, as described by them in one instance, was to add 5 drops of serum from a guinea-pig to 1 drop of infected blood from a mouse and 1 drop of citrate solution. The mixture was incubated for 4 hours.

Rosenthal (1918) showed that, when mice infected with nagana were treated with tartar emetic and 3 to 48 hours later were again re-infected with a massive dose of the trypanosomes, no infection resulted. In his view, this provided indisputable evidence that immune bodies appeared in the serum within a short time of the commencement of the chemotherapeutic treatment. The fact that a drug was less effective prophylactically than therapeutically was, according to him, also an indication in support of his view. He uses the expression 'phenomenon paradox' of divergence ('paradoxe Divergenz') of prophylactic and curative action of tartar emetic. As further evidence, he cites the fact that no retardation in the curative process occurred when the action of the emetic was partially neutralized by the injection of potassium hexatantalate. The weakening effect of the latter on tartar emetic was evidenced by the fact that, when mice were given a combination of these 2 drugs, they died of the infection in the same manner as controls.

Mayer and Zeiss (1921*a*) showed that suramin could be demonstrated in the blood of animals for 5 months after injection and that the curative effect of the serum varied in inverse ratio to the length of the interval between drug treatment and withdrawal of the serum. These authors also found that the serum thus withdrawn from human subjects treated with suramin for sleeping sickness had a curative effect on animals infected with *T. brucei*, *T. equinum*, *T. equiperdum* and *T. rhodesiense*.

It would appear that immune serum from animals suffering from chronic or subacute trypanosomiasis, while possessing protective properties, is ineffective against the trypanosomes occurring in the animals themselves. Van Saceghem (1922*b*) gives a possible explanation why this is so. According to him, these trypanosomes, as they arise, vaccinate themselves with the antibodies and some of them may escape destruction, being protected by the specific antibodies. He (1922*c*) also postulates the production of what he calls 'obstructive' ('empéchente') in the blood of goats that have recovered from a chronic *T. ruandae* infection. He states that this substance prevents the trypanosomes from multiplying, eventually resulting in recovery of the animals. He claims to have successfully developed a method of serum therapy on the basis of these observations. When recently infected goats are repeatedly given subcutaneous injections of large doses of serum from an animal that has been infected with *T. ruandae* for a long time, 'obstructive' is produced in its blood whereby the disease develops into a chronic form: the animal progresses to recovery and become strongly resistant to the infection. Furthermore, blood from such recovered goats, when given in doses of 500 to 1000 c.c., has a curative effect on goats recently infected with *T. ruandae*. Further elucidating his views, Van Saceghem (1922*d*) states that in chronic *T. ruandae* infection the majority of the trypanosomes are destroyed by the antibodies but that a proportion of them—apparently those that vaccinate them selves—turn into neurotropic forms and, being incapable of living in the circulation (for reasons not clear), migrate to the cerebrospinal fluid. Referring to the precise function of the antibodies, he (1922e) says that they do not operate against the trypanosomes but against the toxins liberated by them.

According to Luengo and De Buen (1924), the trypanocidal action of serum after drug treatment may be partially due to the destruction of the trypanosomes, so that the object of treatment—e.g. with suramin—should not be complete sterilization of the blood but of progressive destruction of the parasites to provoke a continuous liberation of the immune bodies.

The serum of normal animals after drug injection has been mentioned by some workers as exerting a protective action against trypanosomes. Thus, Ledentu (1926) found that 1 c.c. of serum from cattle that had been infected 48 hours previously with suramin protected guinea-pigs against *T. gambiense* infection. A dose of 0·2 c.c. of the serum did not even lengthen the incubation period, although it retarded the rate of progress of the disease. It would, however, seem probable that the protective effect in such cases is attributable to the drug present in the serum, for, as is well known, suramin and its equivalents are slowly excreted. Ledentu found that the protection was not strong enough to prevent infection when large quantities of infective blood were injected.

While no appreciable difference appears to have been recorded between the nature of protection afforded by the serum from normal animals on the one hand and that from infected animals on the other, Nattan-Larrier and Noyer (1932) showed that the anti-trypanosome substances in the former were ultra-filtrable ('laissent passer la substance protectrice et la substance curative'), but that those in the latter were incapable of passing through a collodion filter. It is more than probable that such fundamental difference in the physical properties of these 2 classes of substances will eventually be found to be reflected in the nature of degree of protection afforded by them. It is of importance that in studies of this kind the methods of testing should be standardized to yield comparable results, for even such a small factor as the age of the serum has been found to be related to its potency. Thus, Taliaferro and Osten (1943) found that the protective action of normal sheep serum against *T. duttoni* infection in mice was usually noticeable in serum which was more than 1 year old—a fact which is suggestive of the possibility that the action in some cases is enhanced by 'ripening'.

CHAPTER XVIII

Immunity in Trypanosomiasis

AS with protozoan diseases generally, solid immunity against trypanosomiasis has so far proved to be impossible to establish. Accounts of extensive trials are on record to devise methods of conferring immunity on animals against the disease, as is evidenced by the fact that Penso (1938) has cited 450 publications directly or indirectly related to the subject. The attainment of this objective does not as yet appear to be in sight. Amongst the early workers on immunity against trypanosomiasis, mention may be made of Lanfranchi (1912), who dealt with the question of trypanolytic power of the spleen. Aoki and Kodama (1913) unsuccessfully attempted immunization against *T. equiperdum* with formalin killed or dried trypanosomes. Schilling and Rondani (1913, *infra*) discussed the relationship between trypanosome toxin and immunity.

It would seem that more precise knowledge of the genesis of pathogenicity of trypanosomes themselves will have to be sought for before any truly rational method of immunization against this form of infection can be developed. It would be of interest to consider here the reason why certain species of trypanosomes are not pathogenic for the host which normally harbour them. According to Delanoë (1911), the natural immunity of the mouse against *T. rotatarium*, *T. noctuae*, *T. scardinii*, *T. phoxini*, *T. theileri*, and *T. vespertilionis* is due to the fact that they are constantly phagocyted in the animal's body. He found that when the cultural flagellates of the parasites are injected intraperitoneally, they are destroyed and disappear very quickly. He ascribes the non-pathogenicity of *T. lewisi* to similar reasons, except for the fact that the phagocytosis of the parasites occurs throughout the rat's body.

A point of importance brought out by Mesnil and Leger (1912*a*) is that resistance to trypanosomiasis may vary in different localities among ruminants, which, in France, have been found by them to develop, as a rule, complete immunity after recovery from trypanosomiasis. The finding emphasizes the importance of keeping controls in immunity experiments to exclude the possibility of resistance in treated animals being due to climatic conditions.

Rosenthal and Spitzer (1924) adopted what, to all appearance, was a good procedure for ascertaining the nature of the substance that imparts protective power to immune serum and the precise role played by the reticulo-endothelial membrane in the process. It was observed by these workers that human serum did not lose its trypanocidal power in animals that had been freed of leucocytes ('aleukö-zytäron tier'). When human blood was freed of leucocytes by treatment with thorium X, the serum lost its trypanocidal power and a somewhat similar effect was produced when the reticulo-endothelial membrane was blocked with iron preparations, while a more pronounced diminution occurred in splenectomized animals and a still greater diminution of the trypanocidal action of the serum took place when the blocking was done in combination with the extirpation of the spleen. According to them, the macrophage system plays an important role in causing human serum to acquire trypanocidal power. They also observed that human serum, which was non-trypanocidal *in vitro*, was activated by the reticulo-endothelial system *in vivo*. According to Kligler and Comaroff (1935), it is due to the activation of the reticulo-endothelial system itself that an increase in immunity results from the injection of dead trypanosomes.

Gradual recovery after contracting trypanosomiasis early in life has been held by certain workers to be capable of making bovines resistant to subsequent infection, but, as will be seen from what has to be stated later, the results so far recorded in regard to this have been inconclusive. On the other hand, there is some evidence to show that adult ruminants, after recovery from surra, often acquire a fairly durable immunity. Thus, Mesnil and Leger (1912*b*) quote the following figures representing the minimum duration of immunity in years acquired by ruminants after recovery; 2 zebus, 3; 2 bovines, 2; 1 goat, 2½. Besides, a goat after suffering from nagana for 1 month is reported by them to have been immune against the disease for about 2 years. Additional evidence of immunity was obtained by the fact that serum from the recovered animals was found to possess protective power. From these observations, they came to the conclusion that under such conditions the animals acquire 'generalement' an 'immunité solide'.

That there is a possibility of erroneously attributing protective power to serum when such power is in reality attributable to the residual effect of a previously injected trypanocidal drug is illustrated by a series of observations recorded by Levaditi and Mutermilch (1913). The serum of rats that had been cured of nagana by salvarsan was found by them to retain its trypanocidal power for 30 hours even after the serum was heated at 56°C. They, therefore, concluded that this was attributable not to the trypanolytic properties of the serum but to the presence of a residual quantity of the drug in it. Further, the liberation of antibodies in the serum could not be demonstrated until after the fifth day of drug inoculation, in spite of the presence of considerable numbers of trypanosomes in it and the richness of the quantity of antigen produced by the action of the drug. They (1914) discuss the relative protective power of 3 kinds of serum: (*a*) serum from rabbits cured by salvarsan; (*b*) serum containing trypanolytic amboceptors from infected rabbits killed at the height of trypanosomiasis; (*c*) serum representing a combination of both (*a*) and (*b*). When tested on mice, the first showed only slight power to affect multiplication of the parasites or to delay death of the infected animals; the second showed likewise a feeble or perhaps slightly better action; the third was effective in preventing multiplication of the parasites, but under the conditions of the experiment, the precise function of the amboceptors* was difficult to estimate, for the presence of salvarsan itself in the serum was a contributory factor in bringing about the result.

Here it should be mentioned that, in the course of his studies on nagana in rats, guinea-pigs and rabbits, Ciuca (1914*b*) found that serum from animals treated by emetic showed no trypanocidal effect *in vitro*: no difference was observed between serum of treated and that of untreated animals whether they had been previously infected or not. On the other hand, when blood withdrawn from infected animals was mixed with emetic, both serum and extracts of the corpuscles immobilized the trypanosomes within 5 minutes. Salvarsan also gave similar results. It would seem that Ciuca considered these results to be attributable to the fact that the drug was incapable, *in vivo*, of provoking the liberation of antibodies in the serum. On the other hand, a possible explanation for this fact is that the drug, after injection, was too quickly excreted to cause the production of antibodies.

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*For the information of field workers not conversant with the terminology used in chemotherapy, it may be mentioned that the conception of amboceptors forms an integral part of the side-chain theory enunciated by Ehrlich as an explanation of the phenomenon of immunity. Known also as an 'immune body' or an 'intermediary body,' the amboceptor is an outlying part (receptor) of the animal cell, which after combining with a foreign substance in the form of food or toxin, has become detached from the cell and is capable, by means of its two so called 'haptophorous' groups, to act as a binding link between an invading cell and normally present complement thereby causing the destruction of the invading cell.

The more important methods whereby the setting up of an immunity against trypanosomiasis in animals has been sought to be achieved by different workers are outlined below.

I. BY THE USE OF VACCINES

As far back as in 1911, attempts were made by Latapie to confer immunity on rats against *T. equinum* by the use of a vaccine. Trypanosomes from infected rats were isolated by centrifugation from the blood, triturated and macerated in serum of infected rats and then used as vaccine. The injection of this vaccine resulted in the incubation period being lengthened by about 4 days. Teichmann and Braun (1911), in commenting on Latapie's findings, remarked that, as no controls had been kept, the resistance shown by the treated animals might have been of a non-specific character, being attributable to the effect of serum only. Mention may also be made here of unsuccessful attempts made by Laveran and Mesnil (cited by Laveran, 1911) to prevent *T. brucei* infection in small animals by the injection of infected blood rendered non-virulent by heating at 40°C. Laveran (1911) himself attempted to vaccinate white mice and guinea-pigs against *T. gambiense* by means of trypanosomes isolated from infected rat's blood and then centrifuged, dried, powdered and mixed with distilled water; the method proved ineffective.

Braun and Teichmann (1912) claim to have successfully immunized small animals against dourine and nagana with a vaccine prepared by centrifuging blood of infected rats, drying the centrifugate and treating with toluol, which, it is stated by them, does not injure the antigen. They observed that, when rabbits were feebly immunized with this vaccine against dourine, they resisted infection for 6 weeks longer than controls but that when, on the appearance of the trypanosomes, they were treated with highly active serum, they failed to react, showing that the trypanosomes had become serum-fast. They made the interesting finding that serum-fast strains were capable of producing antigen, but that the antibodies produced were effective against serum-fast strains only and not against the original strains, namely, those that had not been subjected to the action of serum.

Some observations made by Novy *et al.* (1912) in regard to the immunological properties of *T. lewisi* deserve passing mention here, for they may be found to hold good for pathogenic trypanosomes also. According to these observations, the plasmolyzed trypanosomes, when injected into rats, make them immune to infection. Living cultures made on rabbit blood agar medium, when given to rats in 1 or more injections, confer on them a solid immunity, probably lasting through life.

Schilling (1912 *a*, *b*, *c*), who worked over a number of years to develop a method of conferring immunity on animals against trypanosomiasis, initially tested the protective power of *T. brucei* for horses when these trypanosomes were killed by means of chemical substances, but were still capable of producing antibodies. The organisms, after being isolated from blood, were treated for 2 hours with 1 : 400 solution of tartar emetic. This mixture was centrifuged and the sediment used for injection. Schilling claims to have obtained immunity by this method, although its duration was inconstant. Two horses were given a series of 7 vaccine injections from a total of 50 rats between 10th April and 25th June. They resisted infection when injected with nagana blood on 1st August. Schilling states that, when infection was not prevented, the incubation period was prolonged from 24 hours to 4 to 27 days. In a proportion of rats, the trypanosomes re-appeared and this was followed by death of the animals, but in many cases a single injection was sufficient to make them resistant to a series of re-infections. The reason why horses and cattle do not develop such resistance is, according to him, due to the fact that no remedy is known that would sterilize them with the same degree of certainty as in small animals. Schilling (1913*a*), in collaboration with Rondani, also attempted to immunize mice by means of trypanotoxins obtained by keeping trypanosomes at 37°C. for different lengths of time. Toxic effects were produced when such trypanosomes were injected in the form of suspension, but some immunity resulted when the trypanosomes were given in an emulsion form. With reference to nagana, these workers (1913*b*) postulate the existence in the bodies of trypanosomes what they call immuno antigen, which, on introduction into susceptible animals, renders them immune against infection with living trypanosomes.

Laveran and Roudsky (1913) made unsuccessful attempts to confer immunity on mice against *T. brucei* infection by the intraperitoneal injection of suspensions of 5 doses of 2 cgm. of the dried and powdered trypanosomes at 5-day intervals. Nattan-Larrier (1913) tested the possibility of conferring immunity on mice by injecting centrifuged trypanosomes combined with bile; the method was unsuccessful.

Darling (1913) used a naturally avirulent strain of *T. hippicum* derived from guinea-pigs in which it had been kept alive by passaging for nearly 300 days. A relatively mild infection developed when it was injected into a mule. After recovery, its immunity was tested by a virulent strain, but the animal survived. According to him, the method involves no danger from a recrudescence' of the infection remaining in a suppressed form in the recovered animal and then regaining its virulence, thereby becoming infective to other animals.

Laveran (1913) claims to have produced immunity by the use of sub-toxic doses of liquids containing trypanosomes and kept for some time at 37°C. Working in collaboration with Marullaz, he (1914) found that nagana trypanosomes remained infective for mice when the trypanosomes were merely subjected to the action of salvarsan.

A series of unsuccessful attempts was made by Hintze (1915) to vaccinate small animals against nagana by means of trypanosomes taken from infected rats, dried a about 39°C., washed and administered intraperitoneally in salt solution. Spleen pulp liver and serum from infected rats were also found to possess little protective power Only by incorporating rat spleen pulp he was able to keep a guinea-pig parasite-free for 9 months. He found it difficult to push ('schwierigkeiten stossen') the large quantity of clean inoculum ('derartiger grosser Menger reinen Impfstoffes') required in such cases.

Ponselle (1923) prepared a vaccine by mixing monobasic and dibasic phosphates with the blood of mice infected with nagana. Mice injected with the vaccine resisted infection when given an inoculation of nagana blood 8 to 10 days later. In discussing the previous efforts of various workers to confer immunity by means of vaccines, Ponselle remarks that practically none of these vaccines had proved successful.

Kligler and Comaroff (1945) found that the results of inoculating dead trypanosomes varied in accordance with the experimental animals employed. In rats, such inoculation resulted in the prolongation of the incubation period and of the disease itself, but the lytic antibody production was negligible; a production of lytic antibody took place in rabbits while nothing in particular was noticeable in guinea-pigs. They further remark : 'An important consideration in the study of trypanosome immunity is the difference in the degree of resistance manifested by different animals and by the same animal at different ages'.

Describing the stages by which immunity develops in calves injected with living trypanosomes, Van Saceghem (1936) states that, when blood from guinea-pigs containing *T. congolense*, is inoculated into a 8-15 day old calf, it develops a benign infection and soon shows a large but fluctuating number of trypanosomes in its circulating blood. After 4 to 6 months, the parasites cease to appear with regularity, with a progressive lengthening of the negative period, eventually the blood remaining completely free from parasites and becoming non-infective on sub inoculation. A series of later experiments carried out by him (1938*b*) showed that when calves under 1 month were inoculated with *T. congolense*, a benign infection resulted, this being followed by recovery and a sterile immunity.

It was observed by Lourie and O'Connor (1937) that the incubation period of *T. rhodesiense* infection in mice increased by 2 days or more in the course of 1,000 passages and that the strains that developed as a result of these passages resisted the lytic action of immune serum from mouse, unlike the original strain. According to these workers, a kind of polyvalent lytic serum against each type of organism may be obtained from a mouse that has been infected with a mixture of several types of parasites. The finding must be regarded as having registered an advance in the formulation of immunity measures against trypanosomiasis inasmuch as it envisages the possibility of conferring protection against all forms of trypanosomiasis by infecting animals with minimal doses (as recommended by Schilling, *infra*) of a combination of several species of trypanosomes.

An interesting possibility of the protective action of heterologous sera is suggested by the observation, recorded by Roubaud and Provost (1941), that when a strain of *T. brucei* was kept *in* prolonged contact *in vitro* with the serum of a goat suffering from chronic *T. vivax* infection, the strain either ceased to be infective for mice or the infection developed after a long incubation period. The possibility of utilizing this method for rendering a strain avirulent for protective inoculation would appear to merit attention.

It would appear that, if immunity against trypanosomiasis proves at all to be capable of being consistently established by methods involving some form of infective inoculation, the inoculum in such cases must consist of living—if avirulent—and not dead trypanosomes. Gallo (1946) records having failed to obtain active immunity against *T. venezuelense* by the use of dried trypanosomes or extracts of the parasites. Kraneveld and Djaemoedin (1949*b*) attempted to immunize horses and guinea-pigs against *T. evansi* by inoculating them with a powder prepared from infected blood but the attempt proved unsuccessful.

II. By Drug Virus Inoculation

As is well known, cases are now and again reported in which cattle, either on natural recovery or on recovery after drug treatment, developed a sterile immunity against trypanosomiasis. Macqueen (1937) mentions 21 such recoveries from *T. vivax* infection, all of these having been subjected to re-infection with *T. vivax*; only 1 of the animals died within an observation period of 1 year, against 8 out of 10 controls. Seventeen of the recovered animals were inoculated with *T. congolense*; only 1 died against 6 out of 10 controls. Briercliffe (1939) reports that, on exposing 11 of a batch of 16 premunized animals in a tsetse area, 6 died within 6 months, 4 were considerably reduced in health and only 1 could be used for work. These findings illustrate the desirability of basing conclusions on the results of trials under field conditions.

It is of interest for workers in India that, in a monograph published as far back as in 1911, Terry recorded the observation that mice could be readily immunized against surra when they were infected with the parasites and then treated with a combination of amidonaphthol disulphonic acid and dichlorbenizidine, this combination being either used alone or in conjunction with acetyl-atoxyl. The mice proved fully resistant to infection when they were tested 6 days after the last treatment. Terry states that the protection due to immunity and that due to unexcreted drugs can be distinguished by the fact that, in the former case, the intraperitoneal injection of a massive dose of virulent trypanosomes fails to result in infection, or, at any rate, the infection is delayed, whereas the presence of unexcreted drug fails to prevent it.

Concerning the question whether the duration of immunity following drug treatment is influenced by the particular trypanocide used, Rudolph (1911), from his experimental observations on *T. brucei* and *T. equinum* infections in white mice, came to the conclusion that it was not so. He also found that an animal immune to infection with one relapse strain might be susceptible to infection with another and that a relapse strain which had been passaged through several animals showed a tendency to regain the properties of the strain from which it originally came.

Sergent and Lhe/ritier (1919) claim to have successfully immunized a camel against dabab' (called by them *T. berberum* infection) by first infecting it, then subjecting it to a curative treatment and finally reinforcing the immunity by re-inoculation of the parasites. They also record a case of immunity acquired by a camel under natural conditions. It is noteworthy that both animals proved susceptible to infection without, however, developing the actual disease symptoms and they, therefore, regarded the animals as having developed what they called 'relative immunity'. That the immunity was not in the nature of pre-munition was shown by the fact that large quantities of blood from them failed to infect when inoculated into healthy animals.

Before ascribing protective power to the serum of recovered animals after drug treatment, it is important, as indicated earlier, to exclude the possibility of this protection being due to the unexcreted portion of the drug itself. A case somewhat in point is the finding made by Mayer and Zeiss (1921*b*) that the activity of the serum in healthy rabbits treated with suramin was not due to any alteration in the blood but to the drug itself bound up with the proteins of the serum. Ledentu (1926) found that it was only when the serum was withdrawn within 48 hours of its treatment with suramin that it could protect a guinea-pig when the latter was injected with the serum simultaneously with *T. gambiense*.

The experimental observations recorded by Kligler and Weitzman (1926) point to the conclusion that methods designed to confer immunity against trypanosomiasis bo the use of dead or attenuated trypanosomes are not likely to prove successful. Working with a strain of *T. evansi*, they found that neither sedimented trypanosomes nor trypanosomes autolyzed by freezing and thawing nor, again, those killed by heating at 56°C. were, on injection, capable of producing immunity in rabbits. On the other hand, such injections sensitized the animals making the incubation period of the disease much shorter than usual, with 'explosive' clinical symptoms. In a second series of experiments, they tested the immunizing power of the trypanosomes when mixed with suramin or when their injection was followed by that of the drug, the latter having been used at 0·005 gm. per kilo.—a dose that had been found insufficient for protection. A control rabbit that received only trypanosomes developed a fatal infection as usual. The rabbits which received suramin after the infective inoculation died of the infection after an incubation period of 10 days. The remaining rabbits recovered after showing some symptoms of the disease but without actually developing it. No notable difference in the results was observed when varying quantities of the drug were used, but in all such cases the results were suggestive of some degree of resistance having been acquired by the animals. Bevan (1936*b*) states that infection followed by treatment with tartar emetic would protect an animal against infection with the same species of trypanosome. He writes that in the course of his 25 years' experience he found that 'treated animals are either sterilized of the parasites and so again become susceptible to re-infection, or are not completely sterilized and become tolerant'. It should be mentioned here that Kligler and Weitzman's results described above are at variance with those of Rao and Muddaliar (1934), who obtained immunity in rats against surra when they were first given infection and then subjected to treatment with suramin.

Browing and Gulbransen (1936) observed that the degree of 'active' immunity to re-infection with *T. brucei* in mice sterilized against this parasite by a single dose of a rapidly excreted drug was 'markedly' determined by the strain of trypanosome employed. Rabbits cured of *T. brucei* infection were found by them to possess a high degree of resistance to re-infection with the same species. Injection of a mixture of living trypanosomes and serum derived from immune rabbits conferred some protection on mice.

The view has been expressed from time to time by a large number of workers that a certain degree of immunity can be set up by infecting animals and then injecting them at an appropriate time with some trypanocidal drug. At the best, such methods can only be of very limited value, for it is common experience that animals that had recovered from trypanosomiasis are susceptible to re-infection after some lapse of time. Duke (cited by Van Hoof *et al.*, 1940 *b*) has postulated the liberation of trypanosome antigen when a human volunteer, after receiving an injection of 1 gm. of suramin, did not develop infection during an observation period of nearly 1 year after being exposed to infective bites of flies. According to Duke, the immune antibody produced by the action of the antigen contributed to the immunizing effect of the drug. Van Hoof and his collaborators failed to obtain confirmation of this view from their trials on guinea-pigs. According to them (1940*c*), the immunity resulting from the combined action of suramin and trypanosomes occurs in a small proportion of animals and is not durable. It would seem more probable that such immunity is maintained only so long as the drug remains in the circulation in trypanocidal concentration. Van Hoof and his collaborators (1940*d*) consider a suitable protective dose of suramin for man to be 0·025 gm. per kilo., or a flat dose of 1·25 to 1·5 gm., repeated every 3 months.

Richet (1942), working in Roubaud's laboratory, discovered a method of what was called by him chemo-biological immunization. It was observed by him that, when rats or rabbits were infected with *T. rhodesiense* and then cured by sulfursenol, it resisted re-infection with the same strain 80 days later, but such immunity only developed when the drug injection was given not before but after the septicaemic stage of the disease. Similar results were obtained with *T. brucei* and in this case a strong immunity was found to have developed after 90 to 140 days. Richet uses the term 'sulfar' for sulfarsenol. Roubaud (1942) states having satisfied himself as to the acceptability of Richet's findings. In collaboration with Caubet, Roubaud (1944) also conducted a series of similar experiments by infecting rats with *T. gambiense* and then successfully treating them with sulfursenol. They found that, whether a septicaemic or a neurotropic strain of *T. gambiense* was used, the period of incubation was greatly prolonged, compared with that of controls, when the rats were subjected to test inoculation. This, however, was not observed when *T. evansi* was used. They confirmed the observation recorded by previous workers that infection followed by recovery after drug treatment resulted in the liberation of large quantities of toxin.

Leonard (1946) does not consider that the full curative dose of drug is essential to produce immunity. According to him, a subcurative dose is capable of altering the metabolism of the parasites in a manner to render them more susceptible to phagocytosis, the process being aided by the immunizing response of the host, although he considers that such immunity is only temporary owing – for reasons not given—to the greater power of resistance acquired by the surviving parasites. Leonard's findings are at the best only of academic interest, for nothing short of a sterilizing dose can be recommended for use in any attempt designed to produce immunity by drug injection.

It is possible that, in some instances the failure to demonstrate immunity in animals after infecting and later treating them with drugs has been due to the test inoculations having been performed too early after the treatment. Such a possibility is suggested by the fact that Fulton and Lourie (1946) found that, when mice were infected with *T. rhodesiense* and *T. congolense* and then treated with diamidinodimethyl stilbene, or with reduced tryparsamide, some degree of immunity to infection with a homologous strain was set up soon after the treatment in 124 out of 128 instances, but absolute immunity could be demonstrated not until after 3 to 4 weeks. The test inoculum used contained 5,000—50,000 trypanosomes.

III. By setting up a State of Premunition

While nothing short of complete recovery is apparently capable of preventing equine surra from progressing to a fatal issue, such need not be the case in infected cattle where, as in India, surra in these animals often exists in a benign form. Although premunition is known to be effective against theileriasis, its possibilities as a protective measure in bovine trypanosomiasis had not been tested until Parkin and Hornby (1930) produced evidence to show that premunition might be successfully practised against *T. congolense* and *T. vivax* infections in cattle. According to them, it is undesirable in these infections to produce complete sterilization. They recommend the conversion by drug treatment of chronic cases to a condition of premunition, although they recognize that the two can be hardly differentiated. According to them, 'it is not difficult to premunize a bovine against a single strain of *T. congolense*'. Perhaps a convenient standard for achieving premunition would be to apply drug treatment up to a stage that would prevent multiplication of the parasites and not destroy them.

Mettam (1940), working in Nigeria, cites Van Saceghem as having shown that a state of 'premunity', although evidently not immunity, may be produced by artificial chronic infection. Mettam himself subjected 8 calves to inoculation with *T. vivax* with the object of setting up a condition of premunity ; 2 of these died of chronic trypanosomiasis. Twenty-four calves received both *T. vivax* and *T. congolense* and death occurred in 9 of these.

Dios and Kuhn (1942) claim to have succeeded in setting up a state of premunition for short periods in a horse and rats by injecting *T. equinum* parasites. The injection caused a slight elevation of temperature with the appearance of trypanosomes in the circulating blood. Although the parasites later disappeared, the blood continued to remain infective for both rats and the horse. When the immunity of the horse was tested by injection of virulent blood, it remained resistant to infection, except for a temporary appearance of a few trypanosomes in its circulating blood.

Some experimental evidence has been brought forward by Fiennes (1947*b*) to show that the protective mechanism of immunity is essentially different from that of premunition. Working with *T. congolense*, he observed that the infectivity of trypanosomes for mice was not affected when they had been incubated for 1 hour with serum from cattle that had, to all appearance, recovered from *T. congolense* infection. On the other hand, serum from a premunized animal exercised a marked protective action. In order to induce absorption of the trypanosome antibody, quantities of the protective sera were incubated with large numbers of trypanosomes. The latter proved non-infective when injected together with the serum, but were infective when they were separated and washed with Ringer-glucose. Fiennes advances the view that in such cases the protection is due to some substance analogous to opsonin, which renders the trypanosomes more susceptible to the action of phagocytes. According to him, it is probable that immunity is brought about by the issues themselves, while premunity is, 'at any rate in part', due to humoral factors, 'of which we have not yet full knowledge'.

It would seem desirable at this point to refer to the distinction between the terms 'premunization', 'sterilization' and 'tolerance' (*infra*). Schilling's method (which will be described presently) has sometimes been called one of premunition, although his objective was evidently to maintain a state of complete freedom from parasites by reinforcing from time to time the immunity set up by an initial infection in the early life of the animal. On the other hand, it is difficult to see how a state of tolerance, as defined by Bevan, differs in any material respects from what is commonly understood by premunition, for both postulate the maintenance of a state of benign infection. In any case, none of these methods can be regarded as of any substantial use as a means of combating animal trypanosomiasis. As Lester (1936) remarks, they may at the best do some occasional good to cattle exposed to infection, but they cannot be depended upon for appreciably influencing mortality among animals.

IV. BY SETTING UP A STATE OF TOLERANCE

In a paper published in 1910, Manteufel expressed the belief that the phenomenon of immunity itself was a form of tolerance, the trypanosomes being still present in the body, although they are not demonstrable either under the microscope or by biological tests, so that the animals may be regarded as 'salted' as in piroplasmosis.

Bevan (1936*a*) envisages the possibility of producing a condition of 'trypanosomiasis tolerance' amongst cattle—an expression by which he means a condition more or less analogous to premunition, this state being achieved by pushing drug treatment to a point where the parasites would be rendered avirulent without being destroyed. In view, however, of the possibility of 'tolerant' animals being carriers, he recognizes the desirability of obtaining sterile immunity by effecting a radical cure. On the other hand, he points out the limitation of this form of immunity in that animals made immune to one species of trypanosome may be susceptible to infection with another species. The setting up of a condition or tolerance has been consistently advocated by Bevan (1936*b*, 1937*a*) as a method of conferring immunity on cattle against *T. congolense* infection in Southern Rhodesia. According to him, this condition may be brought about by treatment of infected animals by antimony to a degree short of producing actual sterilization and then by periodically reinforcing this tolerance by the bites of infected flies or by the inoculation of parasites.

Bevan (1937*b*) criticizes the present methods of control of animal trypanosomiasi as being localized and palliative and, in his view, 'international action' is necessary to control modern methods of transportation of animals. He found that complete sterilization resulted in the animals being susceptible to re-infection when returned to tsetse areas, so that, according to him, it is tolerance that is to be aimed at. For successful immunization, Bevan mentions the possibility of utilizing the method of cultivating trypanosomes *in vitro*, as developed by Brutstaert and Henrard (1936), who also found that strains of trypanosomes thus cultivated retained their pathogenicity for 156 days.

Bevan (1936*b*) gives the reasons that led him to believe that, by setting up a state of tolerance to trypanosomiasis, cattle might be enabled to live their normal span of life in a fly area. These reasons are briefly as follows : (1) Experienced hunters and explorers in tsetse-infected localities hold the belief that the reason why wild animals are resistant to trypanosomiasis is that they become infected early in life ; (2) tartar emetic having proved to be an effective drug for the treatment of bovine trypanosomiasis in Southern Rhodesia over a number of years, this drug appeared to Bevan to be a suitable one for use—apparently in 'subtrypanocidal' doses—for setting up a state of tolerance against trypanosomiasis ; (3) it was observed that animals which had been completely sterilized of the parasites by this drug became again susceptible to infection, while the incompletely sterilized animals became tolerant ; (4) Bevan's 25 years' experience has shown that such tolerant animals remain healthy in fly areas ; (5) experiments have shown that the young of mice and rats are susceptible to infection, but if they are properly nourished and undisturbed, they sometimes survive an infection with a virulent strain of trypanosomes which would kill adults ; on recovery, they become susceptible to infection again, but if they are re-infected before they have recovered, they develop a tolerance to future infection. Bevan, however, found that the tolerance was liable to break down under adverse field conditions. An abridged version of Bevan's view occurs in a report issued by Brain (1937) on behalf of a Trypanosomiasis Committee.

In elucidating further his theory of immunity through tolerance, Bevan (1940) expresses the view that such tolerance under natural conditions can be set up in both man and animals, although he recognizes the danger of their serving as carriers. From the results of his experiments, he was led to conclude that a lasting immunity could be produced in infected animals by subjecting them to a course of suramin treatment and then reinforcing the immunity by subsequent re-infection. Bevan stresses the desirability of producing a drug that would be effective against all species of trypanosomes.

V. By Infecting Animals Carly in Life

Schilling, a zealous advocate of this method, investigated it over a period of 30 years. Although the method has since proved to be of little value as a means of conferring immunity against trypanosomiasis, the results of his investigation are of sufficient interest to merit consideration in some detail.

Schilling *et al.* (1933) have consistently adhered to the view that immunity from trypanosomiasis in game in Africa is due to infection having been acquired by them at birth, the immunity being reinforced by repeated bites of flies throughout life*. In attempting to simulate this condition, he infected 2 calves with *T. brucei* and 2 foals with *T. congolense* and subjected them to bites of infected flies to maintain the infection and resistance. The animals did not suffer impairment of health as a result of this procedure.

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* Although Schilling has repeated this view in several of his articles, it should be of interest to reproduce the exact statement made by him 'Sils resistent aux trypanosomiases, cela tient a une immunité ou tolérance acquise durant le jeune âge et reinforce édurant toute la vie par les piqures des tsétsés

Schilling *et al.* (1933, 1934) state the reasons that led them to regard recovery after an infection early in life as being likely to confer a durable immunity on animals. These reasons are mainly based on the hypothesis that wild animals, e.g. antelopes, become infected when young and the immunity acquired by them after recovery is repeatedly reinforced by the infective bites of tsetse flies. According to them (1934*a*), "this results not in a 'sterile' immunity, but in a 'labile' infection". In their German paper, they (1934*b*) likewise stress the importance of the infection being acquired by the animals early in life ('frühester Jugend'). They explain the disappointing results of some of their experiments on immunity in cattle against *T. brucei* as having been due to the fact that the strains of trypanosomes used by them were not virulent. They describe a series of more successful experiments carried out by them in Berlin, in 1930-32, on 2 foals with a dry antigen of a virulent strain of *T. congolense.* The protocols of their experiments in a summarized form are given below:

16.X.30	Foal born.
24.X.30	Subcutaneous injection of antigen.
3.XI.30 and 28.XI.30	Subjected to bites of tsetse flies infected with *T. congolense* Irregular fever up to 2.V.32 and trypanosomes up to 23.II.32.
20.IX.32	Blood negative for mice.
22.IX.32	Foal subjected to infective tsetse bites. This resulted in fever and appearance of trypanosomes. Three series of further tsetse bites caused only slight reaction. The second foal was also dealt with in a similar manner and reacted as the first foal did.

The same authors (1934) tested a new procedure for giving initial infection to calves. Instead of using a dry antigen, they injected into a foal and 6 calves only a few trypanosomes from an animal that had developed a labile infection. This was found to give rise to the production of antibodies without demonstrable trypanosomes in the circulation or without significant elevation in temperature, but nevertheless trypanosome antibodies could be demonstrated in the blood 57 days after the infection. In the case of the foal, trypanosomes were invariably detected in the blood, but the temperature was irregular and lasted for a few days only. Five months after the infection, the foal was subjected to bites of *G. morsitans* infected with *T.brucei* and *T.congolense*. A week later, its blood showed both species of trypanosomes, but *T.congolense*, although not *T.brucei*, eventually disappeared. The disappearance of *T.congolense*, according to the authors, points to the conclusion that the initial infection of the foal with *T.congolense* blood had produced a labile infection and this prevented the fly-borne organisms of this species from obtaining a foothold in the animal. What would appear to be substantially the same observations were also published independently by Schilling (1934) and he (1935*a*, *b*, *c*, 1936) gives the results of trials carried out by him on these lines under field conditions in Tanganyika. It would appear from his paper published in 1936, that Schilling made as many as 69 deductions from his experiments. As foals were not obtainable in this locality, Schilling used only calves for his experiments, the 2 species of trypanosomes against which they were immunized being *T.congolense* and *T.brucei*. In the first series of experiments, each inoculum consisted of not more than 50 trypanosomes; if no reaction was noticeable within a fortnight, the injection was repeated. The immunized animals, which were 23 in number, were then sent to a tsetse area, and 13 (57 per cent) were alive after 7 months. For various reasons, Schilling considers only 12 of the 23 calves as having been subjected to conditions answering the requirements of his experiments, so that the actual mortality figure was only 1 out of 12 (9 per cent).

For immunizing a second group of calves, Schilling used a vaccine from rats showing swarming trypanosomes. The blood was defibrinated and dried in a shallow dish; as no sterile precautions were taken, the material on subcutaneous injection often caused suppuration. An emulsion was prepared by mixing 1 part of the dried blood with 10 parts of sterile water and 10 to 20 c.c. of the emulsion was used for a dose. Two calves were given only 1 injection of the vaccine and were subjected to bites of infected flies 22 days later; 1 calf died in about 4 months and the other in about 9 months. Nine out of 10 calves that had been injected with 4 doses of the vaccine at fortnightly intervals were alive after 7 months. A third group of 5 calves were first subjected to infective bites, and on appearance of trypanosomes in their circulating blood, they were each twice injected with half the curative dose of antimosan; 4 of the calves died. There were 13 control calves and death occurred in 8 (61 per cent) of these. All of a batch of 31 non-premunized cows became infected, 16 of these having died in 7 months. In the final analysis of the results of his trials, Schilling puts down the percentage of survivals among premunized animals at 70 to 91, against 39 among the non-premunized. The results are considered by him to be suggestive of the value of this form of premunition. He emphasizes the deisrability of premunizing only calves in good condition and he further recommends that it should be done both against *T.congolense* snd *T.vivax*. He considers the method of premunization by injecting small numbers of live trypanosomes as preferable to the use of vaccine, for it approximates the conditions obtaining in nature. Some additional account of Schilling's experiments is given later.

Schilling (1936*a*) discusses the theoretical implications of the methods of premunization advocated by him. He gives the results of a series of experiments to show that trypanosome cells possessing similar antigenic property may differ in the possession of receptors (in the specific sense of receptivity for antibody). Mice that had been infected with a normal strain and then cured were refractory to a homologous normal strain but susceptible to a serum-fast strain. On the other hand, mice that had been infected with a serum-fast strain and then cured were refractory to both normal and serum-fast strains. This showed that both strains possessed similar antigenic properties but differed in respect of receptors. He emphasizes the possiblity that field strains of the same species of trypanosome from different localities may likewise undergo changes in respect of antigenic properties and receptors. Schilling discusses the conditions under which such changes may be brought about.

Schilling's view concerning natural immunity of most animals against trypanosomiasis being due to infection acquired early in life is shared by Hornby *et al.* (1934), at least in so far as immunity against *T.theileri* infection is concerned. The fact that cattle in Tanganyika are immune to *T.theileri* is ascribed by them to this cause. To forestall the possiblity of fatal *T.theileri* infection being acquired by cattle in *theileri*-free localities during anti-rinderpest inoculations, these workers suggest the prior imunization against this infection or using, as until recently had been done in India, rinderpest virus from goats, or swabbing animals with the virus, instead of inoculating it.

Van Saceghem (1934*b*) tested Schilling's method of immunization by infecting 4 calves, 2 to 15 days old, with *T. congolense*. Two died, probably due, according to him, to causes other than trypanosomiasis. Van Saceghem considers his experiments to have been too few to admit of any conclusions being drawn. He, however, points out that the idea of conferring immunity on some stage in the development of the animal by provoking infection early in life and that of conferring immunity on a much later stage in the life of the animal are two different concepts In his paper published in 1938(*b*), Van Saceghem favours the method of imuunization of cattle recommended by Schilling and suggests that a suitable age at which cattle might be immunized against *T.congolense* is 1 month. Immunized animals were found by him to resist infection by blood inoculation or when kept in areas frequented by *Stomoxys* flies, but their resistance in tsetse areas does not appear to have been tested.

Schilling *et al.* (1934) adopted the following procedure for immunizing horses against *T.congolense* infection. As foals, the horses were given a 'basal immunity' by the inoculation of trypanosome antigen or they were first infected and then administered a subcurative dose of antimosan or, again, they were given a 'primary infection' from a latent form of the disease. They were then repeatedly subjected to infective bites of tsetse flies. The method of immunity was claimed by them to have been successful. Schilling and his collaborators (1934) continued their immunity observations in Germany with tsetse flies imported there from Tanganyika. The flies were infected with *T.brucei* and *T.congolense* and the experimental observations were made on young horses and cattle. Besides confirming their previous findings about the success of early inoculations, they concluded that immunity was also obtainable if the infective inoculation was done from chronic cases. Schilling (1935*d*) mentions having found that about 40 per cent of young calves in Tanganyika were highly resistant to trypanosomiasis, this according to him being presumably attributable to immune mothers. On this assumption, Schilling injected 18 calves either with a minimal dose of living trypanosomes or with a vaccine prepared from dead trypanosomes; 6 of these died after contracting disease resulting from such inoculations. Schilling (1935*b*) followed up his experiments in Tanganyika by giving the calves an initial immunity and then keeping them in a fly zone where nagana existed in a virulent form. It was found by him that a dose of 50 trypanosomes (*T.congolense* and *T.brucei*) sometimes failed to produce infection until the animal concerned was given a third inoculation of the dose. When animals thus initially immunized were subjected to fly bites, 57 per cent of them survived after 7 months' exposure, against 38 per cent of the controls. The possibility of immunizing in the fœtal stage by infecting pregnant cows was also tested with inconclusive results.

Schilling would appear to have given wide publicity to his findings on immunity trials (1936*b*, *c*, *d*, *e*), apparently in the belief that the method developed by him offered a lasting solution of the question of bovine trypanosomiasis in Africa. He (1936*b*) writes: 'In all my experiments executed since 1899 it is proved : (1) that young animals (foals, calves) in the first weeks of their life possess a considerable resistance against the African trypanosomes transmissible by tsetse flies; (2) that this natural resistance can be enhanced to full immunity against super-infection through the fly either by minimal infections or by preventive vaccine treatment'. For 'srtong resistance' he uses the expression' 'beträchtliche Resistanz'. In another paper, he (1936*f*) states that some 2 per cent of the 'animaux jeunes' possess considerable resistance against trypanosomiasis. It is probable that this belief seemed to him to be particularly warranted by the fact that, of a batch of 12 calves infected 4 times by injections of 10 to 50 trypanosomes each and then kept in fly areas, 9 (66 per cent) were alive after 23 months, against, 3 (23 per cent) of 13 controls.

Schilling *et al.* (1936), summarize the observations on these immunity test as follows: (1) Calves immunized by minimal doses of living trypanosomes or with driod blood of infected rats proved more resistant than controls when they were kept in a fly zone, but the injection of living trypanosomes sometimes caused heavy mortality. (2) While antibody production by trypanosomes was irregular (non-motile trypanosomes were sometimes infective and motile ones non-infective) the production of antibody was demonstrated by the fact that suspensions of trypanosomes, when mixed with serum, were non-infective on inoculation. (3) A single trypansone isolated from an old strain produced infection. (4) As between trypanosomes from a late and those from an early stage of the disease, the inoculation of the former resulted in a longer incubation period and shorter period of survival. (5) Successive inoculations into mice of progressively increasing numbers of *T.congolense* trypanosomes—commencing from 20 and rising in the survivors to 200—resulted in the final survival of 4 out of a batch of 23. None survived in the controls. Schilling (1936 *f*) gives an analysis of the results of his experiments on immunization and exposure of calves to tsetse bites in Tanganyika and in Germany.

The results of the immunization method adopted by Schilling cannot be regarded as having been encouraging, for in the third report of his work he (1936*b*) gives the number of deaths as follows when the supposedly immunized calves were placed in fly zones: 6 out of 10 vaccinated with dried blood of rats infected with *T. brucei* and *T. congolense*; 9 out of 17 that received minimal doses of trypanosomes; 3 survived out of a batch of 12 calves kept as controls.

From time to time, Schilling (1939*a*, *b*) reported upon the conditions of the animals kept in fly areas. Throughout his observations he held the view that the method of immunization adopted by him, although not completely successful, might be expected to save a fair proportion of animals from dying of trypanosomiasis. In one of his papers, he (1939*a*) states that, out of 11 calves that received minimal doses of *T. congolense*, 6 were alive after 4 to 6 years of exposure in a fly belt, while 2 survived out of 9 calves immunized with killed trypanosomes; 13 were kept as controls and only 1 out of these was alive. In support of his view that resistance to trypanosomiasis was acquired from immune mothers, Schilling cited the instances of calves born in tsetse areas and successfully reared to maturity.

In a paper published in (1941*b*), Hornby gives an account of the ultimate fate of the animals premunized by Schilling either by the minimal dose method or by the use of vaccine. From this account, it would appear that almost all the animals had died by the time Hornby recorded his observations. He, therefore, concludes that none of the protective methods so far recommended is likely to prove of value in tsetse areas in Africa. He further writes: 'Schilling thought he had devised a method of immunization that after reinforcement would be of great practical value, but the subsequent history of the animals which he thought were really immune has shown that his conclusions were premature and his high hopes were not justified'.

Schilling's view concerning the possibility of conferring immunity on cattle by giving them an infective inoculation early in life was based upon his belief that the natural immunity against trypanosomiasis observed amongst game in Africa was produced in a similar way. Against this view, Kleine (1942) points out that sheep and goats in Africa are also naturally immune without being infected when young. The controversy was continued in two further communications (Schilling, 1942; Kleine, 1943) without unequivocal evidence having been produced in support of either view. Fiennes (1948) considers a 'defeatist' view—such as that advanced by Kleine—to be hardly warranted. He states having observed a condition of natural resistance in various categories of animals, such as: adult wild animals, although the young were susceptible; a proportion of cattle exposed to bites of tsetse flies; young cattle under laboratory conditions; cattle persistently subjected to drug treatment against trypanosomiasis. It is of interest that, according to an unpublished communication by Carmichael (cited by Fiennes) young of game animals, notably duiker and sitatunga, in tsetse areas are as susceptible as domestic animals to artificial infection with *T. congolense*, so that immunity in such cases is acquired and not hereditary.

VI. Hereditary Immunity

Minning (1936) records an instance of only 34 becoming infected on inoculation out of a batch of 45 young rats born of *T. lewisi*-infected mother, while all of a batch of 16 others born of uninfected mother became infected on inoculation. He found that the immunity against *T. lewisi* was not acquired either through the placenta or through the act of suckling ('plazentar oder durch den Saugakt'). Bevan (1936*b*) found that infection with *T. rhodesiense* could not be effected *in utero*.

Van Saceghem (1936) records having obtained a progressive reduction of trypanosomes in 16 calves when they were inoculated with a strain of *T. congolense* before they attained the age of 16 days. The circulating blood of these calves became completely free from the parasites in 4 to 6 months.

Evidence of hereditary immunity against *T. lewisi* infection has been furnished by Culbertson (1938). This worker found that rats born of mothers recently cured of *T. lewisi* infection were immune to infection with these parasites for the first few weeks of their life and they were also immune when nourished by foster mothers susceptible to *T. lewisi* infection. Further, rats born of susceptible mothers proved resistant to infection when they had been raised by immune foster mothers. Culbertson (1941) found that immunity through milk was also transmitted by mother rats that had been immunized by a vaccine consisting of a suspension of about 4 million *T. lewisi* per c.c. of 0·5 per cent formolized saline solution. The vaccine was used in doses of 0·1 c.c. at 3-day intervals, a total of 8 such injections being given. 'Such immunity as the young may acquire through placental inoculation is largely lost within a few days after birth'.

Gonzalez (1942) states that the progeny of untreated *T. equiperdum*-infected mice showed some degree of immunity for 2 months after birth. It would seem worthy of investigation whether this age was not the optimum for the immunizing inoculations recommended by Schilling, although his method of conferring immunity did not fulfil the hopes that had seemed warranted by the results of his initial observations.

VII. 'Local Immunity'

The Japanese worker, Murakawa (1930*a*, *b*) uses the expression 'local immunity' in trypanosomiasis, presumably meaning immunity localized in a particular area in the body of the host. He claims to have produced such immunity in mice, rats and guinea pigs by the intraperitoneal injection of vaccines prepared from *T. equiperdum*, *T. brucei*, and *T. gambiense.* He states that this immunity later develops into a general immunity, He found that, while feeding of dead trypanosomes did not produce immunity, living trypanosomes, whether sensitized with bile or not, prevented abdominal infection in animals but it did not give rise to general immunity.

To sum up, a method of setting up a durable immunity to trypanosomiasis does not appear to be in sight. Kleine (1941) views with disfavour any of the methods so far advocated for immunizing cattle against trypanosomiasis, whether by the use of parasites rendered avirulent by passaging, or by setting up a state of tolerance by antimony treatment and then exposing animals to bites of tsetse, or by giving infection to cattle during their calfhood. He points out that these methods may at best enable animals to resist infection by local strains of trypanosomes but not by more virulent strains or by other species of trypanosomes. He even concludes that cattle-raising in Africa is not likely to prove successful in tsetse areas. This view is shared by Hornby (1941*b*), who, referring to the ultimate fate of Schilling's immunized animals, states that only one of these was alive in 1941.

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